

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 March 2002 (28.03.2002)

PCT

(10) International Publication Number
WO 02/24234 A2

(51) International Patent Classification⁷: **A61K 48/00**

(21) International Application Number: **PCT/US01/29480**

(22) International Filing Date:
20 September 2001 (20.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/665,493 20 September 2000 (20.09.2000) US

(71) Applicant (for all designated States except US): **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MANNING, William, C., Jr.** [US/US]; 3660 Country Club Drive, Redwood City, CA 94061 (US). **DWARKI, Varavani, J.** [US/US]; 16 Grove Farm Road, Pittstown, NJ 08867 (US). **RENDAHL, Katherine** [US/US]; 1221 Grizzly Peak Boulevard, Berkeley, CA 94708 (US). **ZHOU, Shangzhen** [CN/US]; 125 Fundy Bay, Alameda, CA

94502 (US). **McGEE, Laura, H.** [US/US]; 652 54th Street, Oakland, CA 94609 (US). **LAU, Dana** [US/US]; Apartment A, 280 Lenox Avenue, Oakland, CA 94610 (US). **FLANNERY, John, G.** [US/US]; 1728 Marin Avenue, Berkeley, CA 94707 (US). **MILLER, Sheldon, S.** [US/US]; 186 Hillcrest Road, Berkeley, CA 94705 (US). **WANG, Fei** [CN/US]; 981 9th Street, Albany, CA 94710 (US). **DIPOLO, Adriana** [VE/CA]; 704 Champagneur Avenue, Outremont, Quebec H2V 3P8 (CA).

(74) Agents: **CULLMAN, Louis, C. et al.**; Oppenheimer Wolff & Donnelly LLP, 840 Newport Center Drive, Suite 700, Newport Beach, CA 92660-7007 (US).

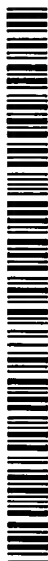
(81) Designated States (national): AU, CA, JP, US.

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/24234 A2

(54) Title: **USE OF RECOMBINANT GENE DELIVERY VECTORS FOR TREATING OR PREVENTING DISEASES OF THE EYE**

(57) Abstract: Gene delivery vectors, such as, for example, recombinant adeno-associated viral vectors, and methods of using such vectors are provided for use in treating or preventing diseases of the eye.

BEST AVAILABLE COPY

USE OF RECOMBINANT GENE DELIVERY VECTORS FOR TREATING OR PREVENTING DISEASES OF THE EYE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Patent Application No. 5 09/525,956, filed March 15, 2000, which application claims priority to U.S. Provisional Application No. 60/124,460, filed March 15, 1999, and U.S. Provisional Application No. 60/174,984, filed January 6, 2000, all of which applications are incorporated by reference in their entirety.

TECHNICAL FIELD

10 The present invention relates generally to compositions and methods for treating diseases of the eye, and more specifically, to the use of various gene delivery vectors which direct the expression of selected gene products suitable for treating or preventing diseases of the eye.

BACKGROUND OF THE INVENTION

15 Eye diseases represent a significant health problem in the United States and world-wide. Presently, over 80 million Americans are affected with potentially blinding eye disease, with 1.1 million being legally blind. Twelve million individuals suffer from some degree of visual impairment that cannot be corrected. The total economic impact of eye disease is also very significant. In 1981, the estimated economic impact of visual 20 impairment on the U.S. economy was 14 billion per year. By 1995, this impact had grown to an estimated 38.4 billion (National Eye Institute NIH).

A wide variety of eye diseases can cause visual impairment, including for example, macular degeneration, diabetic retinopathies, inherited retinal degeneration such as retinitis pigmentosa, glaucoma, retinal detachment or injury and retinopathies (whether 25 inherited, induced by surgery, trauma, a toxic compound or agent, or, photically).

One structure in the eye that can be particularly affected by disease is the retina. Briefly, the retina, which is found at the back of the eye, is a specialized light-sensitive tissue that contains photoreceptor cells (rods and cones) and neurons connected to a neural network for the processing of visual information (see Figure 10). This information
5 is sent to the brain for decoding into a visual image.

The retina depends on cells of the adjacent retinal pigment epithelium (RPE) for support of its metabolic functions. Photoreceptors in the retina, perhaps because of their huge energy requirements and highly differentiated state, are sensitive to a variety of genetic and environmental insults. The retina is thus susceptible to an array of diseases that
10 result in visual loss or complete blindness.

Retinitis pigmentosa (RP), which results in the destruction of photoreceptor cells, the RPE, and choroid typify inherited retinal degenerations. This group of debilitating conditions affects approximately 100,000 people in the United States.

A great deal of the progress made in addressing this important clinical
15 problem has depended on advances in research on photoreceptor cell biology, molecular biology, molecular genetics, and biochemistry over the past two decades. Animal models of hereditary retinal disease have been vital in helping unravel the specific genetic and biochemical defects that underlie abnormalities in human retinal diseases. It now seems clear that both genetic and clinical heterogeneity underlie many hereditary retinal diseases.

20 The leading cause of visual loss in the elderly is Age-related Macular Degeneration (AMD). The social and economic impact of this disease in the United States is increasing. The macula is a structure near the center of the retina that contains the fovea. This specialized portion of the retina is responsible for the high-resolution vision that permits activities such as reading. The loss of central vision in AMD is devastating.
25 Degenerative changes to the macula (maculopathy) can occur at almost any time in life but are much more prevalent with advancing age. With growth in the aged population, AMD will become a more prevalent cause of blindness than both diabetic retinopathy and glaucoma combined. Laser treatment has been shown to reduce the risk of extensive macular scarring from the "wet" or neovascular form of the disease. The effects of this

treatment are short-lived, however, due to recurrent choroidal neovascularization. Thus, there are presently no effective treatments in clinical use for the vast majority of AMD patients.

Other diseases of the eye, such as glaucoma, are also major public health problems in the United States. In particular, blindness from glaucoma is believed to impose significant costs annually on the U.S. Government in Social Security benefits, lost tax revenues, and healthcare expenditures.

Briefly, glaucoma is not a uniform disease but rather a heterogeneous group of disorders that share a distinct type of optic nerve damage that leads to loss of visual function. The disease is manifest as a progressive optic neuropathy that, if left untreated, leads to blindness. It is estimated that as many as 3 million Americans have glaucoma and, of these, as many as 120,000 are blind as a result. Furthermore, it is the number one cause of blindness in African-Americans. Its most prevalent form, primary open-angle glaucoma, can be insidious. This form usually begins in midlife and progresses slowly but relentlessly. If detected early, disease progression can frequently be arrested or slowed with medical and surgical treatment.

Glaucoma can involve several tissues in the front and back of the eye. Commonly, but not always, glaucoma begins with a defect in the front of the eye. Fluid in the anterior portion of the eye, the aqueous humor, forms a circulatory system that brings nutrients and supplies to various tissues. Aqueous humor enters the anterior chamber via the ciliary body epithelium (inflow), flows through the anterior segment bathing the lens, iris, and cornea, and then leaves the eye via specialized tissues known as the trabecular meshwork and Schlemm's canal to flow into the venous system. Intraocular pressure is maintained *vis-à-vis* a balance between fluid secretion and fluid outflow. Almost all glaucomas are associated with defects that interfere with aqueous humor outflow and, hence, lead to a rise in intraocular pressure. The consequence of this impairment in outflow and elevation in intraocular pressure is that optic nerve function is compromised. The result is a distinctive optic nerve atrophy, which clinically is characterized by excavation and cupping of the optic nerve, indicative of loss of optic nerve axons.

Primary open-angle glaucoma is, by convention, characterized by relatively high intraocular pressures believed to arise from a blockage of the outflow drainage channel or trabecular meshwork in the front of the eye. However, another form of primary open-angle glaucoma, normal-tension glaucoma, is characterized by a severe optic neuropathy in the absence of abnormally high intraocular pressure. Patients with normal-tension glaucoma have pressures within the normal range, albeit often in the high normal range. Both these forms of primary open-angle glaucoma are considered to be late-onset diseases in that, clinically, the disease first presents itself around midlife or later. However, among African-Americans, the disease may begin earlier than middle age. In contrast, juvenile open-angle glaucoma is a primary glaucoma that affects children and young adults. Clinically, this rare form of glaucoma is distinguished from primary open-angle glaucoma not only by its earlier onset but also by the very high intraocular pressure associated with this disease. Angle-closure glaucoma is a mechanical form of the disease caused by contact of the iris with the trabecular meshwork, resulting in blockage of the drainage channels that allow fluid to escape from the eye. This form of glaucoma can be treated effectively in the very early stages with laser surgery. Congenital and other developmental glaucomas in children tend to be severe and can be very challenging to treat successfully. Secondary glaucomas result from other ocular diseases that impair the outflow of aqueous humor from the eye and include pigmentary glaucoma, pseudoexfoliative glaucoma, and glaucomas resulting from trauma and inflammatory diseases. Blockage of the outflow channels by new blood vessels (neovascular glaucoma) can occur in people with retinal vascular disease, particularly diabetic retinopathy.

Primary open-angle glaucoma can be insidious. It usually begins in midlife and progresses slowly but relentlessly. If detected, disease progression can frequently be arrested or slowed with medical and surgical treatment. However, without treatment, the disease can result in absolute irreversible blindness. In many cases, even when patients have received adequate treatment (e.g., drugs to lower intraocular pressure), optic nerve degeneration and loss of vision continues relentlessly.

The present invention provides compositions and methods for treating and preventing a number of diseases of the eye, including for example, retinal diseases and degenerations such as RP and AMD, as well as other diseases such as neovascular disease. The present invention also provides other, related advantages.

5 SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for treating, preventing, or, inhibiting diseases of the eye. Within one aspect of the present invention, methods are provided for treating or preventing diseases of the eye comprising the step of intraocularly administering a gene delivery vector which directs the expression
10 of one or more neurotrophic factors, or, anti-angiogenic factors, such that the disease of the eye is treated or prevented. Within related aspects of the present invention, gene delivery vectors are provided which direct the expression of one or more neurotrophic factors such as FGF, as well as gene therapy vectors which direct the expression of one or more anti-angiogenic factors. Within certain embodiments of the invention, a viral promoter (*e.g.*,
15 CMV) or an inducible promoter (*e.g.*, tet) is utilized to drive the expression of the neurotrophic factor.

Representative examples of gene delivery vectors suitable for use within the present invention may be generated from viruses such as retroviruses (*e.g.*, FIV or HIV), herpesviruses, adenoviruses, adeno-associated viruses, and alphaviruses, or from non-viral
20 vectors.

Utilizing the methods and gene delivery vectors provided herein a wide variety of diseases of the eye may be readily treated or prevented, including for example, glaucoma, macular degeneration, diabetic retinopathies, inherited retinal degeneration such as retinitis pigmentosa, retinal detachment or injury and retinopathies (whether inherited,
25 induced by surgery, trauma, a toxic compound or agent, or, photically). Similarly, a wide variety of neurotrophic factors may be utilized (either alone or in combination) within the context of the present invention, including for example, NGF, BDNF, CNTF, NT-3, NT-4, FGF-2, FGF-5, FGF-18, FGF-20 and FGF-21.

Within certain embodiments of the invention, it is preferred that the gene delivery vector be utilized to deliver and express an anti-angiogenic factor for the treatment, prevention, or, inhibition of diabetic retinopathy, wet AMD, and other neovascular diseases of the eye (*e.g.*, ROP). Within other embodiments it is desirable that
5 the gene delivery vector be utilized to deliver and express a neurotrophic growth factor to treat, prevent, or, inhibit diseases of the eye, such as, for example, glaucoma, retinitis pigmentosa, and dry AMD. Within yet other embodiments, it may be desirable to utilize either a gene delivery vector which expresses both an anti-angiogenic molecule and a neurotrophic growth factor, or two separate vectors which independently express such
10 factors, in the treatment, prevention, or inhibition of an eye disease (*e.g.*, for diabetic retinopathy).

Within further embodiments of the invention, the above-mentioned methods utilizing gene delivery vectors may be administered along with other methods or therapeutic regimens, including for example, photodynamic therapy (*e.g.*, for wet AMD),
15 laser photocoagulation (*e.g.*, for diabetic retinopathy and wet AMD), and intraocular pressure reducing drugs (*e.g.*, for glaucoma).

Also provided by the present invention are isolated nucleic acid molecules comprising the sequence of Figure 2, vectors which contain, and/or express this sequence, and host cells which contain such vectors.

20 Within further aspects of the present invention gene delivery vectors are provided which direct the expression of a neurotrophic factor, or, an anti-angiogenic factor. As noted above, representative examples of neurotrophic factors include NGF, BDNF, CNTF, NT-3, NT-4, FGF-2, FGF-5, FGF-18, FGF-20 and FGF-21. Representative examples of anti-angiogenic factors include soluble Flt-1, soluble Tie-2 receptor, and
25 PEDF. Representative examples of suitable gene delivery vectors include adenovirus, retroviruses (*e.g.*, HIV or FIV-based vectors), alphaviruses, AAV vectors, and naked DNA vectors.

Within yet other aspects of the invention non-human animal models of neovascular diseases of the eye are provided, comprising an animal having an angiogenic

(i.e., pro-angiogenic) transgene in the eye. Within various embodiments, the neovascularization may be retinal or choroidal neovascularization. Within other embodiments, the animal may be a mouse or rat. As noted herein, a wide variety of angiogenic transgenes may be utilized to generate the non-human animal model, including
5 for example, angiogenic transgenes that encode VEGF and/or an angiopoietin such as angiopoietin-1.

Also provided are methods of making such non-human animal models comprising the general steps of administering to a non-human animal a gene delivery vector which directs the expression of an angiogenic transgene. As noted above, a wide
10 variety of gene delivery vectors (e.g., rAV and rAAV) can be utilized, as well as nucleic acid molecules which encode the angiogenic transgene (e.g., nucleic acid molecules encoding VEGF or angiopoietin). Within certain embodiments, the gene delivery vector can be administered subretinally or intravitreally. Within further embodiments the animal model can be utilized as a model for Age-related Macular Degeneration (AMD), diabetic
15 retinopathy, or, retinopathy of prematurity (ROP).

Also provided are methods for determining the ability of an anti-angiogenic factor to inhibit neovascularization of the eye, comprising the general steps of (a) administering to an animal model as described herein an anti-angiogenic factor, and (b) determining the ability of the anti-angiogenic factor to inhibit neovascularization of the
20 eye. As noted herein, the anti-angiogenic factor may be administered by a variety of routes, including for example, topically, subretinally, or, intravitreally. Further, the animal model may be utilized to test the efficacy of drugs, compounds, or other factors or agents for a wide variety of eye-related neovascular diseases (including AMD and ROP).

These and other aspects of the present invention will become evident upon
25 reference to the following detailed description and attached drawings. In addition, various references are set forth herein which describe in more detail certain procedures or compositions (e.g., plasmids, etc.), and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of pKm201bFGF-2.

Figures 2A and 2B are the nucleic acid sequence of pKm201bFGF-2 (SEQ I.D. No. ____).

5 Figure 3 is a schematic illustration of pD10-CMV-FGF-5.

Figure 4 is a western analysis of FGF-5 rAAV infected 293 cells.

Figure 5 is a schematic illustration of pD10-CMV-FGF-5 (sig-).

Figure 6 is a western analysis of pD10-CMV-FGF-5 (sig-) transfected 293 cells.

10 Figure 7 is a schematic illustration of pD10-CMV-FGF-18.

Figure 8 western analysis of 293 cells transfected pD10-CMV-FGF-18.

Figures 9A and 9B are photographs which show that blue-gal staining is visible across 40% of a retina transfected with AAV-CMV-lacZ. All photoreceptors appear to express lacZ at the injection site, except at the edge where individual cells are visible.

15 Figure 10 is a schematic illustration which shows the retina within the eye, and the organization of cells within the retina.

Figures 11A and 11B are photographs of wild-type and degenerated S334ter rat retinas. S334ter is a rat model for retinitis pigmentosa.

20 Figures 12A, 12B and 12C are photographs of degenerated S334ter, FGF-2 injected S334ter and PBS injected S334ter rat retinas. As can be seen in these figures, FGF-2 injected into the S334ter rat retina substantially slows the progression of disease.

Figure 13 is a graph which plots Outer Nuclear Layer (ONL) thickness for FGF-2 subretinally injected, PBS subretinally injected, and an uninjected control.

Figure 14 is a bar graph which plots ONL thickness at p60.

25 Figures 15A, 15B and 15C are photographs of FGF-2 expressing cells stained with an anti-FGF-2 antibody.

Figures 16A and 16B are photographs which show gene delivery to cells in the ganglion cell layer following intraocular injection of recombinant rAAV-CMV-lacZ. a) superior quadrant of a retinal flat-mount processed for Blue-Gal staining to visualize AAV-

infected neurons. Notice the large number of axons converging at the optic nerve head (asterisk). b) Retinal radial section showing the AAV-mediated LacZ gene product in cells of the ganglion cell layer. A large number of these cells can be identified as RGCs because of the intense LacZ staining in axons projecting to the optic nerve head (asterisk). RPE: 5 retinal pigment epithelium, PS: photoreceptor segments, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer. Scale bars: a) 0.5 mm, b) 50 μ m.

Figure 17 is a graph which shows the time-course of AAV-mediated transgene expression in the ganglion cell layer of the adult rat retina. A recombinant AAV 10 vector (rAAV-CMV-lacZ) was injected into the vitreous chamber of adult rats and 2, 4 and 8 weeks later, Lac-Z positive neurons in the ganglion cell layer (GCL) were counted in retinal flat-mounts. The values are the mean of 3-4 retinas per time point \pm standard deviation ($p < 0.001$).

Figures 18A and 18B are photographs which show the localization of the 15 AAV-mediated LacZ gene product in retrogradely labeled RGCs. a) Retinal radial section showing LacZ immunopositive RGCs transduced with AAV (excitation 520-560, barrier 580, emission 580); b) Same section examined under a different fluorescent filter (excitation 355-425, barrier 460, emission 470) to visualize RGCs backlabeled with Fluorogold from the superior colliculus. Notice that the vast majority of LacZ 20 immunopositive neurons are also labeled with Fluorogold. An exception, a LacZ positive cell that is not Fluorogold labeled, is shown (arrow), and could represent a displaced amacrine cell or RGC that did not incorporate the retrograde tracer. INL: inner nuclear layer, IPL: inner plexiform layer; GCL: ganglion cell layer. Scale bar: 10 μ m.

Figure 19 is a graph which quantifies Fluorogold- and LacZ-positive cells in 25 the ganglion cell layer following intravitreal injection of rAAV-CMV-lacZ. The number of Fluorogold-positive cells (FG+) was compared to the number of cells that expressed both the Fluorogold and LacZ markers (FG+, LacZ+) and the number of cells expressing only the reporter gene (LacZ+). Values represent the mean of 4-5 retinal radial sections per animal ($n=4$) \pm standard deviation (S.D.) ($p < 0.001$).

Figure 20 is a photograph which shows the localization of the heparan sulfate (HS) proteoglycan receptor, the cellular receptor for AAV, in the intact adult rat retina. Retinal cryosection immunostained with a polyclonal antibody against the heparan sulfate (HepSS-1; diluted 1:200) followed by biotinylated anti-rabbit Fab fragment, avidin-
5 biotin-peroxidase reagent (ABC Elite Vector Labs, Burlingame, CA). The section was reacted in a solution containing 0.05% diaminobenzidine tetrahydrochloride (DAB) and 0.06% hydrogen peroxide in PB (pH 7.4) for 5 min. Notice the strong labeling in RGCs in the ganglion cell layer (GCL). RPE: retinal pigment epithelium, PS: photoreceptor segments, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer,
10 IPL: inner plexiform layer. Scale bar: 50 μ m.

Figure 21 is a schematic illustration of pD10-VEGF_{UC}.

Figure 22A and 22B are the nucleotide sequence of pD10-VEGF_{UC}.

Figure 23 is a bar graph which shows pD10-VEGF_{UC} rAAV virus infection of 293 cells.

15 Figure 24 is a three dimensional bar-graph which shows VEGF secretion by hRPE after infection with VEGF AAV.

Figure 25 is a three dimensional bar-graph which shows VEGF secretion by hRPE after infection with VEGF AV

20 Figure 26 is a three dimensional bar-graph which shows resistance of hRPE after infection with VEGF AV.

Figure 27 is a schematic illustration of pD10-sFlt-1.

Figure 28A and 28B provide the nucleotide sequence of pD10-sFlt-1.

Figure 29 is the nucleotide sequence of FGF-20.

Figure 30 is the nucleotide sequence of FGF-21.

25 Figure 31 is a schematic illustration of pD10K-FGF-2Sc.

Figure 32A and 32B are the nucleotide sequence of pD10K-FGF-2Sc.

Figure 33 is a graph that compares ONL thickness (μ m) after injection of various vectors into the eye.

Figure 34 is a bar graph that shows inhibition of HMVEC proliferation by sFlt-1 rAAV.

Figures 35A and 35B are photographic images showing retinal blood vessels from a live animal, before sacrifice.

5 Figures 36A, 36B, 36C, and 36D are a series of images showing epoxy sections of an eye at various distances from an AAV-VEGF injection site.

Figures 37A, 37B, 37C, and 37D are photographs which show lectin / BrdU double-staining of the rat retina.

10 Figures 38A and 38B are bar graphs which show sFlt-1 and PEDF rescue of ERGs.

Figure 39 is a graph which shows the ERG of a test and control eye of sFlt-1 treated rats.

DETAILED DESCRIPTION OF THE INVENTION

15

DEFINITIONS

Prior to setting forth the invention, it may be helpful to an understanding thereof to first set forth definitions of certain terms that will be used hereinafter.

"Gene delivery vector" refers to a construct which is capable of delivering, and, within preferred embodiments expressing, one or more gene(s) or sequence(s) of
20 interest in a host cell. Representative examples of such vectors include viral vectors, nucleic acid expression vectors, naked DNA, and certain eukaryotic cells (*e.g.*, producer cells).

"Recombinant adeno-associated virus vector" or "rAAV vector" refers to a gene delivery vector based upon an adeno-associated virus. The rAAV vectors, should
25 contain 5' and 3' adeno-associated virus inverted terminal repeats (ITRs), and a transgene or gene of interest operatively linked to sequences which regulate its expression in a target cell. Within certain embodiments, the transgene may be operably linked to a heterologous

promoter (such as CMV), or, an inducible promoter such as (tet). In addition, the rAAV vector may have a polyadenylation sequence.

"Neurotrophic Factor" or "NT" refers to proteins which are responsible for the development and maintenance of the nervous system. Representative examples of neurotrophic factors include NGF, BDNF, CNTF, NT-3, NT-4, and Fibroblast Growth Factors.

"Fibroblast Growth Factor" or "FGF" refers to a family of related proteins, the first of which was isolated from the pituitary gland (see Gospodarowicz, D., *Nature*, 249:123-127, 1974). From this original FGF (designated basic FGF) a family of related proteins, protein muteins, and protein analogs have been identified (see, e.g., U.S. Patent Nos. 4,444,760, 5,155,214, 5,371,206, 5,464,774, 5,464,943, 5,604,293, 5,731,170, 5,750,365, 5,851,990, 5,852,177, 5,859,208, and 5,872,226), all of which are generally referred to as Fibroblast Growth Factors within the context of the present invention.

"Anti-angiogenic Factor" refers to a factor or molecule which is able to inhibit the proliferation of vascular growth. A variety of assays may be utilized to assess the anti-angiogenic activity of a given molecule, including for example, the assay provided in Example 15, which measures HMVEC (human dermal microvascular endothelial cell) proliferation. Representative examples of anti-angiogenic factors include for example, Angiostatin, 1,25-Di-hydroxy-vitamin D₃, Endostatin, IGF-1 receptor antagonists, Interferons alpha, beta and gamma, Interferon gamma-inducible protein IP-10, Interleukin 1 alpha and beta, Interleukin 12, 2-Methoxyestradiol, PEDF, Platelet factor 4, Prolactin (16kd fragment), Protamin, Retinoic acid, Thrombospondin-1 and 2, Tissue inhibitor of metalloproteinase-1 and -2, Transforming growth factor beta, anti-VEGF antibodies (which should be understood to include fragments of antibodies such as a single chain antibodies, Fab fragments, or, CDR regions), soluble Tie-2 receptor, soluble Tie-2 receptor, soluble Flt-1 and Tumor necrosis factor - alpha.

"Diseases of the Eye" refers to a broad class of diseases wherein the functioning of the eye is affected due to damage or degeneration of the photoreceptors; ganglia or optic nerve; or neovascularization. Representative examples of such diseases

include macular degeneration, diabetic retinopathies, inherited retinal degeneration such as retinitis pigmentosa, glaucoma, retinal detachment or injury and retinopathies (whether inherited, induced by surgery, trauma, a toxic compound or agent, or, photically).

As noted above, the present invention provides compositions and methods
5 for treating, preventing, or, inhibiting diseases of the eye, comprising the general step of administering intraocularly a recombinant adeno-associated viral vector which directs the expression of one or more neurotrophic factors, such that the disease of the eye is treated or prevented. In order to further an understanding of the invention, a more detailed discussion is provided below regarding (A) gene delivery vectors; (B) Neurotrophic Factors; (C) Anti-
10 angiogenic factors; and (D) methods of administering the rAAVs in the treatment or prevention of diseases of the eye.

A. Gene Delivery Vectors

1. Construction of retroviral gene delivery vectors

Within one aspect of the present invention, retroviral gene delivery vectors
15 are provided which are constructed to carry or express a selected gene(s) or sequence(s) of interest. Briefly, retroviral gene delivery vectors of the present invention may be readily constructed from a wide variety of retroviruses, including for example, B, C, and D type retroviruses as well as spumaviruses and lentiviruses (see RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Such retroviruses may be readily obtained
20 from depositories or collections such as the American Type Culture Collection ("ATCC"; Rockville, Maryland), or isolated from known sources using commonly available techniques.

Any of the above retroviruses may be readily utilized in order to assemble or construct retroviral gene delivery vectors given the disclosure provided herein, and
25 standard recombinant techniques (*e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Kunkel, *PNAS* 82:488, 1985). In addition, within certain embodiments of the invention, portions of the retroviral gene

delivery vectors may be derived from different retroviruses. For example, within one embodiment of the invention, retrovirus LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

5 Within one aspect of the present invention, retrovector constructs are provided comprising a 5' LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences, an origin of second strand DNA synthesis and a 3' LTR, wherein the vector construct lacks *gag/pol* or *env* coding sequences.

Other retroviral gene delivery vectors may likewise be utilized within the
10 context of the present invention, including for example EP 0,415,731; WO 90/07936; WO 91/0285, WO 9403622; WO 9325698; WO 9325234; U.S. Patent No. 5,219,740; WO 9311230; WO 9310218; Vile and Hart, *Cancer Res.* 53:3860-3864, 1993; Vile and Hart, *Cancer Res.* 53:962-967, 1993; Ram et al., *Cancer Res.* 53:83-88, 1993; Takamiya et al., *J. Neurosci. Res.* 33:493-503, 1992; Baba et al., *J. Neurosurg.* 79:729-735, 1993 (U.S. Patent
15 No. 4,777,127, GB 2,200,651, EP 0,345,242 and WO91/02805).

Packaging cell lines suitable for use with the above described retrovector constructs may be readily prepared (see U.S. Serial No. 08/240,030, filed May 9, 1994; see also U.S. Serial No. 07/800,921, filed November 27, 1991), and utilized to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant
20 vector particles.

2. Recombinant Adeno-Associated Virus Vectors

As noted above, a variety of rAAV vectors may be utilized to direct the expression of one or more desired neurotrophic factors. Briefly, the rAAV should be comprised of, in order, a 5' adeno-associated virus inverted terminal repeat, a transgene or
25 gene of interest operatively linked to a sequence which regulates its expression in a target cell, and a 3' adeno-associated virus inverted terminal repeat. In addition, the rAAV vector may preferably have a polyadenylation sequence.

Generally, rAAV vectors should have one copy of the AAV ITR at each end of the transgene or gene of interest, in order to allow replication, packaging, and efficient integration into cell chromosomes. The ITR consists of nucleotides 1 to 145 at the 5' end of the AAV DNA genome, and nucleotides 4681 to 4536 (*i.e.*, the same sequence) at the 3' end of the AAV DNA genome. Preferably, the rAAV vector may also include at least 10 nucleotides following the end of the ITR (*i.e.*, a portion of the "D region").

Within preferred embodiments of the invention, the transgene sequence will be of about 2 to 5 kb in length (or alternatively, the transgene may additionally contain a "stuffer" or "filler" sequence to bring the total size of the nucleic acid sequence between the two ITRs to between 2 and 5 kb). Alternatively, the transgene may be composed of same heterologous sequence several times (*e.g.*, two nucleic acid molecules which encode FGF-2 separated by a ribosome readthrough, or alternatively, by an Internal Ribosome Entry Site or "IRES"), or several different heterologous sequences (*e.g.*, FGF-2 and FGF-5, separated by a ribosome readthrough or an IRES).

Recombinant AAV vectors of the present invention may be generated from a variety of adeno-associated viruses, including for example, serotypes 1 through 6. For example, ITRs from any AAV serotype are expected to have similar structures and functions with regard to replication, integration, excision and transcriptional mechanisms.

Within certain embodiments of the invention, expression of the transgene may be accomplished by a separate promoter (*e.g.*, a viral promoter). Representative examples of suitable promoters in this regard include a CMV promoter, RSV promoter, SV40 promoter, or MoMLV promoter. Other promoters that may similarly be utilized within the context of the present invention include cell or tissue specific promoters (*e.g.*, a rod, cone, or ganglia derived promoter), or inducible promoters. Representative examples of suitable inducible promoters include tetracycline-response promoters ("Tet", see, *e.g.*, Gossen and Bujard, *Proc. Natl. Acad. Sci. USA.* 89:5547-5551, 1992; Gossen et al., *Science* 268, 1766-1769, 1995; Baron et al., *Nucl. Acids Res.* 25:2723-2729, 1997; Blau and Rossi, *Proc. Natl. Acad. Sci. USA.* 96:797-799, 1999; Bohl et al., *Blood* 92:1512-1517, 1998; and Haberman et al., *Gene Therapy* 5:1604-1611, 1998), the ecdysone system (see *e.g.*, No et

al., *Proc. Natl. Acad. Sci. USA*. 93:3346-3351, 1996), and other regulated promoters or promoter systems (see, e.g., Rivera et al., *Nat. Med.* 2:1028-1032, 1996;).

The rAAV vector may also contain additional sequences, for example from an adenovirus, which assist in effecting a desired function for the vector. Such sequences
5 include, for example, those which assist in packaging the rAAV vector in adenovirus-associated virus particles.

Packaging cell lines suitable for producing adeno-associated viral vectors may be readily accomplished given readily available techniques (see e.g., U.S. Patent No. 5,872,005).

10 Particularly preferred methods for constructing and packaging rAAV vectors are described in more detail below in Examples 1, 2, 3, and 4.

3. Alphavirus delivery vectors

The present invention also provides a variety of Alphavirus vectors which may function as gene delivery vectors. For example, the Sindbis virus is the prototype
15 member of the alphavirus genus of the togavirus family. The unsegmented genomic RNA (49S RNA) of Sindbis virus is approximately 11,703 nucleotides in length, contains a 5' cap and a 3' poly-adenylated tail, and displays positive polarity. Infectious enveloped Sindbis virus is produced by assembly of the viral nucleocapsid proteins onto the viral genomic RNA in the cytoplasm and budding through the cell membrane embedded with
20 viral encoded glycoproteins. Entry of virus into cells is by endocytosis through clathrin coated pits, fusion of the viral membrane with the endosome, release of the nucleocapsid, and uncoating of the viral genome. During viral replication the genomic 49S RNA serves as template for synthesis of the complementary negative strand. This negative strand in turn serves as template for genomic RNA and an internally initiated 26S subgenomic RNA.
25 The Sindbis viral nonstructural proteins are translated from the genomic RNA while structural proteins are translated from the subgenomic 26S RNA. All viral genes are expressed as a polyprotein and processed into individual proteins by post translational

proteolytic cleavage. The packaging sequence resides within the nonstructural coding region, therefore only the genomic 49S RNA is packaged into virions.

Several different Sindbis vector systems may be constructed and utilized within the present invention. Representative examples of such systems include those
5 described within U.S. Patent Nos. 5,091,309 and 5,217,879, and PCT Publication No. WO 95/07994.

4. Other viral gene delivery vectors

In addition to retroviral vectors and alphavirus vectors, numerous other viral vectors systems may also be utilized as a gene delivery vector. Representative examples of
10 such gene delivery vectors include viruses such as pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch et al., *PNAS* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330 and 5,017,487; WO 89/01973); SV40 (Mulligan et al., *Nature* 277:108-114, 1979); influenza virus (Luytjes et al., *Cell* 59:1107-1113, 1989; McMichael et al., *N. Eng.*
15 *J. Med.* 309:13-17, 1983; and Yap et al., *Nature* 273:238-239, 1978); herpes (Kit, *Adv. Exp. Med. Biol.* 215:219-236, 1989; U.S. Patent No. 5,288,641); HIV (Poznansky, *J. Virol.* 65:532-536; 1991); measles (EP 0 440,219); Semliki Forest Virus, and coronavirus, as well as other viral systems (e.g., EP 0,440,219; WO 92/06693; U.S. Patent No. 5,166,057). In addition, viral carriers may be homologous, non-pathogenic(defective), replication
20 competent virus (e.g., Overbaugh et al., *Science* 239:906-910, 1988), and nevertheless induce cellular immune responses, including CTL.

5. Non-viral gene delivery vectors

In addition to the above viral-based vectors, numerous non-viral gene delivery vectors may likewise be utilized within the context of the present invention.
25 Representative examples of such gene delivery vectors include direct delivery of nucleic acid expression vectors, naked DNA alone (WO 90/11092), polycation condensed DNA linked or unlinked to killed adenovirus (Curiel et al., *Hum. Gene Ther.* 3:147-154, 1992),

DNA ligand linked to a ligand with or without one of the high affinity pairs described above (Wu et al., *J. of Biol. Chem* 264:16985-16987, 1989), nucleic acid containing liposomes (e.g., WO 95/24929 and WO 95/12387) and certain eukaryotic cells (e.g., producer cells - see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial
5 No. 07/800,921).

B. Neurotrophic Factors

As noted above, the term neurotrophic factor refers to proteins which are responsible for the development and maintenance of the nervous system. Representative examples of neurotrophic factors include NGF, BDNF, CNTF, NT-3, NT-4, and Fibroblast
10 Growth Factors.

Fibroblast Growth Factor refers to a family of related proteins, the first of which was isolated from the pituitary gland (see Gospodarowicz, D., *Nature*, 249:123-127, 1974). From this original FGF (designated basic FGF) a family of related proteins, protein mutants, and protein analogs have been identified (see, e.g., U.S. Patent Nos. 4,444,760,
15 5,155,214, 5,371,206, 5,464,774, 5,464,943, 5,604,293, 5,731,170, 5,750,365, 5,851,990, 5,852,177, 5,859,208, and 5,872,226; see generally Baird and Gospodarowicz, *D. Ann N.Y. Acad. Sci.* 638:1, 1991. The first two members of the family to be identified were acidic fibroblast growth factor (aFGF/FGF-1) and basic fibroblast growth factor (bFGF/FGF-2). Additional members of the FGF family include: i-nt-2/FGF-3, (Smith et al., *EMBO J.* 7:
20 1013, 1988); FGF-4 (Delli-Bovi et al., *Cell* 50: 729, 1987); FGF-6 (Marics et al., *Oncogene* 4: 335 (1989); keratinocyte growth factor/FGF-7, (Finch et al., *Science* 245: 752, 1989); FGF-8 (Tanaka et al., *Proc. Natl. Acad. Sci. USA* 89: 8928, 1992); and FGF-9 (Miyamoto et al., *Mol. Cell Biol.* 13: 4251, 1993).

FGF-5 was originally isolated as an oncogene. See Goldfarb et al. US
25 Patent Nos. 5,155,217 and 5,238,916, Zhan et al. "Human Oncogene Detected by a Defined Medium Culture Assay" (*Oncogene* 1:369-376, 1987), Zhan et al. "The Human FGF-5 Oncogene Encodes a Novel Protein Related to Fibroblastic Growth Factors" (*Molecular*

and *Cellular Biology* 8:3487-3495, 1988), and Bates et al. "Biosynthesis of Human Fibroblast Growth Factor 5": (*Molecular and Cellular Biology* 11:1840-1845, 1991):

Other FGFs include those disclosed in U.S. Patent Nos. 4,444,760, 5,155,214, 5,371,206, 5,464,774, 5,464,943, 5,604,293, 5,731,170, 5,750,365, 5,851,990, 5 5,852,177, 5,859,208, and 5,872,226. 5,852,177, and 5,872,226, as well as FGF-20 (U.S. Provisional Application No. 60/161,162) and FGF-21 (U.S. Provisional Application No. 60/166,540).

C. Anti-Angiogenic Factors

A wide variety of anti-angiogenic factors may also be expressed from the
 10 gene delivery vectors of the present invention, including for example, Angiostatin (O'Reilly et al., *Cell* 79:315-328, 1994; O'Reilly et al., *Nat. Med.* 2:689-92, 1996; Sim et al., *Cancer Res.* 57:1329-34, 1997), 1,25-Di-hydroxy-vitamin D₃ (Shibuya et al., *Oncogene* 5:519-24, 1990; Oikawa et al., *Eur. J. Pharmacol.* 178:247-50, 1990; and 182:616, 1990), Endostatin (O'Reilly et al., *Cell* 88:277-85, 1997), Interferons alpha and beta (Sidky et al., *Cancer Res.*
 15 47:5155-61, 1987; Singh et al., *Proc. Natl. Acad. Sci. USA* 92:4562-6, 1995), Interferon gamma (Friesel et al., *J. Cell. Biol.* 104:689-96, 1987), IGF-1 receptor antagonists, Interferon gamma-inducible protein IP-10 (Arenberg et al., *J. Exp. Med.* 1996;184:981-92; Strieter et al., *J. Leukoc. Biol.* 1995;57:752-62; Angiolillo et al., *J. Exp. Med.* 182:155-62, 1995), Interleukin 1alpha and beta (Cozzolino et al., *Proc. Natl. Acad. Sci. USA* 87:6487-
 20 91, 1990), Interleukin 12 (Kerbel and Hawley, *J. Natl Cancer Inst.* 87:557-9, 1995; Majewski et al., *J. Invest. Dermatol* 106:1114-8, 1996; Voest et al., *J. Natl Cancer Inst.* 87:581-6, 1995), 2-Methoxyestradiol (Fotsis et al., *Nature* 368:237-9, 1994), Platelet factor 4 (Taylor and Folkman, *Nature* 297:307-12, 1982; Gengrinovitch et al., *J. Biol. Chem* 270:15059-65, 1995), Prolactin (16kd fragment) (Clapp et al., *Endocrinology* 133:1292-9,
 25 1993; Ferrara, *Endocrinology* 129:896-900, 1991), Protamin, Retinoic acid (Lingen et al., *Lab. Invest* 74:476-83, 1996), Thrombospondin-1 and 2 (Lawler, *Blood*, 67:1197-209, 1986; Raugi and Lovett, *Am. J. Pathol* 129:364-72, 1987; Volpert et al., *Biochem. Biophys. Res. Commun* 217:326-32, 1995), Tissue inhibitor of metalloproteinase-1 and -2 (Moses

and Langer, *J. Cell Biochem* 47:230-5, 1991; Ray and Stetler-Stevenson, *Eur. Respir. J.* 7:2062-72, 1994), Transforming growth factor beta (RayChaudhury, *J. Cell. Biochem* 47:224-9, 1991; Roberts et al., *Proc. Natl Acad. Sci USA* 83:4167-71, 1986), and Tumor necrosis factor – alpha (Frater-Schroeder et al., *Proc. Natl. Acad. Sci. USA* 84:5277-81, 5 1987; Leibovich et al., *Nature* 329:630-2, 1987).

Other anti-angiogenic factors that can be utilized within the context of the present invention include VEGF antagonists such as soluble Flt-1 (Kendall and Thomas, *PNAS* 90: 10705, 1993), pigment epithelium-derived factor or "PEDF" (Dawson et al., *Science* 285:245, 1999), and Ang-1 antagonists such as soluble Tie-2 receptor (Thurston 10 et al., *Science* 286:2511, 1999; see also, generally Aiello et al., *PNAS* 92:10457, 1995; Robinson et al., *PNAS* 93:4851, 1996; Seo et al., *Am. J. Pathol.* 154:1743, 1999).

The ability of a given molecule to be "anti-angiogenic" can be readily assessed utilizing a variety of assays, including for example, the HMVEC assay provided in Example 15.

15 D. Method for Treating and/or Preventing Diseases of the Eye, and Pharmaceutical Compositions

As noted above, the present invention provides methods which generally comprise the step of intraocularly administering a gene delivery vector which directs the expression of one or more neurotrophic factor to the eye, or an anti-angiogenic factor to the 20 eye in order to treat, prevent, or inhibit the progression of an eye disease. As utilized herein, it should be understood that the terms "treated, prevented, or, inhibited" refers to the alteration of a disease course or progress in a statistically significant manner. Determination of whether a disease course has been altered may be readily assessed in a variety of model systems, discussed in more detail below, which analyze the ability of a 25 gene delivery vector to delay, prevent or rescue photoreceptors, as well as other retinal cells, from cell death.

1. Diseases of the eye

A wide variety of diseases of the eye may be treated given the teachings provided herein. For example, within one embodiment of the invention gene delivery vectors are administered to a patient intraocularly in order to treat or prevent macular
5 degeneration. Briefly, the leading cause of visual loss in the elderly is macular degeneration (MD), which has an increasingly important social and economic impact in the United States. As the size of the elderly population increases in this country, age related macular degeneration (AMD) will become a more prevalent cause of blindness than both diabetic retinopathy and glaucoma combined. Although laser treatment has been shown to
10 reduce the risk of extensive macular scarring from the "wet" or neovascular form of the disease, there are currently no effective treatments for the vast majority of patients with MD.

Within another embodiment, gene delivery vectors can be administered to a patient intraocularly in order to treat or prevent an inherited retinal degeneration. One of
15 the most common inherited retinal degenerations is retinitis pigmentosa (RP), which results in the destruction of photoreceptor cells, and the RPE. Other inherited conditions include Bardet-Biedl syndrome (autosomal recessive); Congenital amaurosis (autosomal recessive); Cone or cone-rod dystrophy (autosomal dominant and X-linked forms); Congenital stationary night blindness (autosomal dominant, autosomal recessive and X-linked forms);
20 Macular degeneration (autosomal dominant and autosomal recessive forms); Optic atrophy, autosomal dominant and X-linked forms); Retinitis pigmentosa (autosomal dominant, autosomal recessive and X-linked forms); Syndromic or systemic retinopathy (autosomal dominant, autosomal recessive and X-linked forms); and Usher syndrome (autosomal recessive). This group of debilitating conditions affects approximately 100,000 people in
25 the United States alone.

As noted above, within other aspects of the invention, gene delivery vectors which direct the expression of a neurotrophic growth factor can be administered to a patient intraocularly in order to treat or prevent glaucoma. Briefly, glaucoma is not a uniform disease but rather a heterogeneous group of disorders that share a distinct type of optic

nerve damage that leads to loss of visual function. The disease is manifest as a progressive optic neuropathy that, if left untreated leads to blindness. It is estimated that as many as 3 million Americans have glaucoma and, of these, as many as 120,000 are blind as a result. Furthermore, it is the number one cause of blindness in African-Americans. Its most prevalent form, primary open-angle glaucoma, can be insidious. This form usually begins in midlife and progresses slowly but relentlessly. If detected early, disease progression can frequently be arrested or slowed with medical and surgical treatment. Representative factors that may be expressed from the vectors of the present invention to treat glaucoma include neurotrophic growth factors such as FGF-2, 5, 18, 20, and, 21.

10 Within yet other embodiments gene delivery vectors can be administered to a patient intraocularly in order to treat or prevent injuries to the retina, including retinal detachment, photic retinopathies, surgery-induced retinopathies, toxic retinopathies, retinopathies due to trauma or penetrating lesions of the eye.

 As noted above, the present invention also provides methods of treating, preventing, or, inhibiting neovascular disease of the eye, comprising the step of administering to a patient a gene delivery vector which directs the expression of an anti-angiogenic factor. Representative examples of neovascular diseases include diabetic retinopathy, AMD (wet form), and retinopathy of prematurity. Briefly, choroidal neovascularization is a hallmark of exudative or wet Age-related Macular Degeneration (AMD), the leading cause of blindness in the elderly population. Retinal neovascularization occurs in diseases such as diabetic retinopathy and retinopathy of prematurity (ROP), the most common cause of blindness in the young.

 Particularly preferred vectors for the treatment, prevention, or, inhibition of neovascular diseases of the eye direct the expression of an anti-angiogenic factor such as, for example, soluble tie-2 receptor or soluble Flt-1.

2. Animal Models

 In order to assess the ability of a selected gene therapy vector to be effective for treating diseases of the eye which involve neovascularization, a novel model for

neovascularization (either choroidal or subretinal) can be generated by subretinal injection of a recombinant virus (e.g., rAV or rAAV) containing an angiogenic transgene such as VEGF and/or angiopoietin. Within certain embodiments, an angiogenic transgene such as angiopoietin-1 can be used in combination with another factor such as VEGF, in order to
5 generate neovascularization. The extent and duration of neovascularization induced by the gene delivery vectors containing an angiogenic transgene such as VEGF can be determined using fundus photography, fluorescein angiography and histochemistry.

To assess the ability of anti-angiogenic molecules to prevent neovascularization in the model described above, a D10- sFlt-1 rAAV (or other gene
10 delivery vector which directs the expression of an anti-angiogenic factor) is intraocularly injected, either by subretinal or intravitreal routes of injection. Generally, subretinal injection of the gene delivery vector may be utilized to achieve delivery to both the choroidal and inner retinal vasculature. Intravitreal injection can be utilized to infect Muller cells and retinal ganglion cells (RGCs), which deliver anti-angiogenic protein to the
15 retinal vasculature. Muller cells span the retina and would secrete the therapeutic protein into the subretinal space.

Such injections may be accomplished either prior to, simultaneous with, or subsequent to administration of an angiogenic factor or gene delivery vector which expresses an angiogenic factor. After an appropriate time interval, inhibition of
20 neovascularization can be determined using fundus photography, fluorescein angiography and/or histochemistry.

While there are many animal models of retinal neovascularization such as oxygen-induced ischemic retinopathy (Aiello et al., PNAS 93: 4881, 1996.) and the VEGF transgenic mouse (Okamoto et al., Am. J. Pathol. 151: 281, 1997), there are fewer models
25 of choroidal neovascularization (e.g., laser photocoagulation as described by Murata et al., IOVS 39: 2474, 1998). Subretinal neovascularization from the retinal rather than choroidal blood supply is also observed in VEGF transgenic animals (Okamoto et al., Am. J. Pathol. 151: 281, 1997). Hypoxic stimulation of VEGF expression is known to correlate with neovascularization in human ocular disease.

The pathologic hallmark of glaucomatous optic neuropathy is the selective death of retinal ganglion cells (RGCs) (Nickells, R.W., *J. Glaucoma* 5:345-356, 1996; Levin, L.A. and Louhab, A., *Arch. Ophthalmol.* 114:488-491, 1996.; Kerrigan, L.A., Zack, D.J., Quigley, H.A., Smith, S.D. and Pease, M.E., *Arch. Ophthalmol.* 115:1031-1035, 5 1997). Recent studies indicate that RGCs die with characteristics of apoptosis after injury to the axons of adult RGCs such as axotomy of the optic nerve (ON), and in glaucoma and anterior ischemic optic neuropathy in humans (Nickells, 1996). Thus, damage to the optic nerve by axotomy is used by many researchers as a model for selective apoptotic cell death of adult RGCs.

10 The loss of RGCs caused by ON transection in adult mammals varies from 50% to more than 90% depending on the techniques used to identify RGCs, the proximity of the lesion to the eye, and the age and species of the animal. For example, in a study in adult rats, in which retrogradely transported tracers were used to distinguish RGCs from displaced amacrine cells (Villegas-Pérez, M.P., Vidal-Sanz, M., Bray, G.M. and Aguayo, 15 A.J., *J. Neurosci.* 8:265-280, 1988). ON transection near the eye (0.5-1 mm) leads to the loss of more than 90% of the RGCs by 2 weeks. In contrast, in adult animals in which the ON was cut nearly 10 mm from the eye, 54% of RGCs survived by 3 months (Richardson, P.M., Issa, V.M.K. and Shemie, S., *J. Neurocytol.* 11:949-966, 1982.).

Briefly, the posterior pole of the left eye and the origin of the optic nerve 20 (ON) are exposed through a superior temporal intraorbital approach. A longitudinal excision of the ON dural sheath is performed. The ON is then gently separated from the dorsal aspect of this sheath and completely transected within the orbit, within 1 mm of the optic disc. Care is taken to avoid damage to the ophthalmic artery, which is located on the inferomedial dural sheath of the ON.

25 RGC survival and death following gene delivery can also be examined using two alternative models of ON injury: 1) ON crush; and (2) increased intraocular pressure. In the first model the ON is exposed, and then clamped at a distance of about one millimeter from the posterior pole using a pair of calibrated forceps as previously described (Li et al., *Invest. Ophthalmol. Vis. Sci.* 40:1004, 1999). In the second model, chronic

moderately elevated intraocular pressure can be produced unilaterally by cauterization of three episcleral vessels as described by Neufeld et al. in *PNAS* 17:9944, 1999).

A variety of animal models can be utilized for photoreceptor degeneration, including the RCS rat model, P23H transgenic rat model, the rd mouse, and the S334ter
5 transgenic rat model.

Briefly, in the S334ter transgenic rat model, a mutation occurs resulting in the truncation of the C-terminal 15 amino acid residues of rhodopsin (a seven-transmembrane protein found in photoreceptor outer segments, which acts as a photopigment). The S334ter mutation is similar to rhodopsin mutations found in a subset
10 of patients with retinitis pigmentosa (RP). RP is a heterogeneous group of inherited retinal disorders in which individuals experience varying rates of vision loss due to photoreceptor degeneration. IN many RP patients, photoreceptor cell death progresses to blindness. Transgenic S334ter rats are born with normal number of photoreceptors. The mutant rhodopsin gene begins expression at postnatal day 5 in the rat, and photoreceptor cell death
15 begins at postnatal day 10-15. In transgenic line S334ter-3, approximately 70% of the outer nuclear layer has degenerated by day 60 in the absence of any therapeutic intervention. The retinal degeneration in this model is consistent from animal to animal and follows a predictable and reproducible rate. This provides an assay for therapeutic effect by morphological examination of the thickness of the photoreceptor nuclear layer
20 and comparison of the treated eye to the untreated (contralateral) eye in the same individual animal.

S334ter rats are utilized as a model for RP as follows. Briefly, S334ter transgenic rats are euthanized by overdose of carbon dioxide inhalation and immediately perfused intracardially with a mixture of mixed aldehydes (2% formaldehyde and 2.5 %
25 glutaraldehyde). Eyes are removed and embedded in epoxy resin, and 1 μ m thick histological sections are made along the vertical meridian. Tissue sections are aligned so that the ROS and Müller cell processes crossing the inner plexiform layer are continuous throughout the plane of section to assure that the sections are not oblique, and the thickness of the ONL and lengths of RIS and ROS are measured. These retinal thickness

measurements are plotted and establish the baseline retinal degeneration rates for the animal model. The assessment of retinal thickness is as follows: briefly, 54 measurements of each layer or structure were made at set points around the entire retinal section. These data were either averaged to provide a single value for the retina, or plotted as a distribution of thickness or length across the retina. The greatest 3 contiguous values for ONL thickness in each retina is also compared in order to determine if any region of retina (*e.g.*, nearest the injection site) showed proportionally greater rescue; although most of these values were slightly greater than the overall mean of all 54 values, they were no different from control values than the overall mean. Thus, the overall mean was used in the data cited, since it was based on a much larger number of measurements.

One particularly preferred line of transgenic rats, TgN(s334ter) line 4 (abbreviated s334ter 4) can be utilized for *in vivo* experiments. Briefly, in this rat model expression of the mutated opsin transgene begins at postnatal day P5 in these rats, leading to a gradual death of photoreceptor cells. These rats develop an anatomically normal retina up to P15, with the exception of a slightly increased number of pyknotic photoreceptor nuclei in the outer nuclear layer (ONL) than in non-transgenic control rats. In this animal model, the rate of photoreceptor cell death is approximately linear until P60, resulting in loss of 40-60% of the photoreceptors. After P60, the rate of cell loss decreases, until by one year the retinas have less than a single row of photoreceptor nuclei remaining.

20 3. Methods of Administration

Gene delivery vectors of the present invention may be administered intraocularly to a variety of locations depending on the type of disease to be treated, prevented, or, inhibited, and the extent of disease. Examples of suitable locations include the retina (*e.g.*, for retinal diseases), the vitreous, or other locations in or adjacent to the eye.

Briefly, the human retina is organized in a fairly exact mosaic. In the fovea, the mosaic is a hexagonal packing of cones. Outside the fovea, the rods break up the close hexagonal packing of the cones but still allow an organized architecture with cones rather

evenly spaced surrounded by rings of rods. Thus in terms of densities of the different photoreceptor populations in the human retina, it is clear that the cone density is highest in the foveal pit and falls rapidly outside the fovea to a fairly even density into the peripheral retina (see Osterberg, G. (1935) Topography of the layer of rods and cones in the human
5 retina. *Acta Ophthalmol.* (suppl.) 6, 1-103; see also Curcio, C. A., Sloan, K. R., Packer, O., Hendrickson, A. E. and Kalina, R. E. (1987) Distribution of cones in human and monkey retina: individual variability and radial asymmetry. *Science* 236, 579-582).

Access to desired portions of the retina, or to other parts of the eye may be readily accomplished by one of skill in the art (see, generally Medical and Surgical Retina:
10 Advances, Controversies, and Management, Hilel Lewis, Stephen J. Ryan, Eds., medical illustrator, Timothy C. Hengst. St. Louis: Mosby, c1994. xix, 534; see also *Retina*, Stephen J. Ryan, editor in chief, 2nd ed., St. Louis, Mo.: Mosby, c1994. 3 v. (xxix, 2559 p.).

The amount of the specific viral vector applied to the retina is uniformly
15 quite small as the eye is a relatively contained structure and the agent is injected directly into it. The amount of vector that needs to be injected is determined by the intraocular location of the chosen cells targeted for treatment. The cell type to be transduced will be determined by the particular disease entity that is to be treated.

For example, a single 20-microliter volume (of 10^{13} physical particle titer/ml
20 rAAV) may be used in a subretinal injection to treat the macula and fovea. A larger injection of 50 to 100 microliters may be used to deliver the rAAV to a substantial fraction of the retinal area, perhaps to the entire retina depending upon the extent of lateral spread of the particles.

A 100-ul injection will provide several million active rAAV particles into
25 the subretinal space. This calculation is based upon a titer of 10^{13} physical particles per milliliter. Of this titer, it is estimated that 1/1000 to 1/10,000 of the AAV particles are infectious. The retinal anatomy constrains the injection volume possible in the subretinal space (SRS). Assuming an injection maximum of 100 microliters, this would provide an

infectious titer of 10^8 to 10^9 rAAV in the SRS. This would have the potential of infecting all of the $\sim 150 \times 10^6$ photoreceptors in the entire human retina with a single injection.

Smaller injection volumes focally applied to the fovea or macula may adequately transfect the entire region affected by the disease in the case of macular
5 degeneration or other regional retinopathies.

Gene delivery vectors can alternately be delivered to the eye by intraocular injection into the vitreous. In this application, the primary target cells to be transduced are the retinal ganglion cells, which are the retinal cells primarily affected in glaucoma. In this application, the injection volume of the gene delivery vector could be substantially larger,
10 as the volume is not constrained by the anatomy of the subretinal space. Acceptable dosages in this instance can range from 25 μ l to 1000 μ l.

4. Assays

A wide variety of assays may be utilized in order to determine appropriate dosages for administration, or to assess the ability of a gene delivery vector to treat or
15 prevent a particular disease. Certain of these assays are discussed in more detail below.

a. Electroretinographic analysis

Electroretinographic analysis can be utilized to assess the effect of gene delivery administration into the retina. Briefly, rats are dark adapted overnight and then in dim red light, then anesthetized with intramuscular injections of xylazine (13 mg/kg) and
20 ketamine (87 mg/kg). Full-field scotopic ERGs are elicited with 10- μ sec flashes of white light and responses were recorded using a UTAS-E 2000 Visual Electrodiagnostic System (LKC Technologies, Inc., Gaithersburg, MD). The corneas of the rats are anesthetized with a drop of 0.5% proparacaine hydrochloride, and the pupils dilated with 1% atropine and 2.5% phenylephrine hydrochloride. Small contact lenses with gold wire loops are
25 placed on both corneas with a drop of 2.5% methylcellulose to maintain corneal hydration. A silver wire reference electrode is placed subcutaneously between the eyes and a ground electrode is placed subcutaneously in the hind leg. Stimuli are presented at intensities of -

1.1, 0.9 and 1.9 log cd m⁻² at 10-second, 30-second and 1-minute intervals, respectively. Responses are amplified at a gain of 4,000, filtered between 0.3 to 500 Hz and digitized at a rate of 2,000 Hz on 2 channels. Three responses are averaged at each intensity. The a-waves are measured from the baseline to the peak in the cornea-negative direction, and b-waves are measured from the cornea-negative peak to the major cornea-positive peak. For quantitative comparison of differences between the two eyes of rats, the values from all the stimulus intensities are averaged for a given animal.

b. Retinal tissue analysis

As described in more detail above and below, retinal tissue analysis can also be utilized to assess the effect of gene delivery administration into the retina.

5. Pharmaceutical Compositions

Gene delivery vectors may be prepared as a pharmaceutical product suitable for direct administration. Within preferred embodiments, the vector should be admixed with a pharmaceutically acceptable carrier for intraocular administration. Examples of suitable carriers are saline or phosphate buffered saline.

DEPOSIT INFORMATION

The following material was deposited with the American Type Culture Collection:

<u>Name</u>	<u>Deposit Date</u>	<u>Accession No.</u>
PKm201bFGF-2	3/11/99	#207160
PD10-Kan-FGF-2-Sc		

20

The above material was deposited by Chiron Corporation with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, telephone 703-365-2700. This deposit will be maintained under the terms of the Budapest

Treaty on the International Recognition of the Deposit of Microorganisms for purposes of Patent Procedure. The deposit will be maintained for a period of 30 years following issuance of this patent, or for the enforceable life of the patent, whichever is greater. Upon issuance of the patent, the deposits will be available to the public from the ATCC without
5 restriction.

This deposit is provided merely as a convenience to those of skill in the art, and is not an admission that a deposit is required under 35 U.S.C. § 112. The nucleic acid sequence of this deposit, as well as the amino acid sequence of the polypeptide encoded thereby, are incorporated herein by reference and should be referred to in the event of an
10 error in the sequence described herein. A license may be required to make, use, or sell the deposited materials, and no such license is granted hereby.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

CONSTRUCTION OF A RAAV VECTOR EXPRESSING FGF-2

5 pKm201CMV is an AAV cloning vector in which an expression cassette, consisting of a CMV immediate early promoter/enhancer and a bovine growth hormone (BGH) polyadenylation site, is flanked by inverted terminal repeat (ITR) sequences from AAV-2. Briefly, pKm201CMV was derived from pKm201, a modified AAV vector plasmid in which the ampicillin resistance gene of pEMBL-AAV-ITR (see Srivastava,
10 (1989) *Proc. Natl. Acad. Sci. USA* 86:8078-8082) had been replaced with the gene for kanamycin resistance. The expression cassette from pCMVlink, a derivative of pCMV6c (see Chapman, *Nucleic Acids Res.* 19:193-198 (1991)) in which the BGH poly A site has been substituted for the SV40 terminator; was inserted between the ITRs of pKm201 to generate pKm201CMV.

15 pKm201bFGF-2 was constructed by cloning the following, in order, into the multiple cloning site of pKm201CMV: the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), the bovine FGF-2 cDNA, and the human growth hormone polyadenylation sequence. The cDNA for FGF-2 has two mutations that change amino acid 121 from serine to threonine and amino acid 137 from proline to serine. The DNA
20 sequence of pKm201bFGF-2 is shown in Figure 2 and the plasmid has been deposited with the American Type Culture Collection (ATCC).

 rAAV vector particles were produced by a triple transfection protocol (*Nucleic Acids Res.* 24:596-601, 1996; *J. Exp. Med.* 179:1867-1875, 1994). Briefly, human embryonic kidney 293 cells, grown to 50% confluence in a 10 layer Nunclon cell factory
25 (Nalge Nunc, Int., Naperville, IL), were co-transfected with 400 µg of helper plasmid pKSrep/cap (*Hum. Gene Ther.* 9:477-485, 1998) 400 µg of vector plasmid, and 800 µg of adenovirus plasmid pBHG10 (Microbix Biosystems, Inc., Toronto, Ontario) using the calcium phosphate co-precipitation method. Forty-eight hours after co-transfection, media

was replaced with IMDM + 10% FBS containing adenovirus type 5 dl312 at a multiplicity of infection (MOI) of 2. Seventy-two hours after infection cells were harvested and resuspended in HEPES buffer (200 ml total) and lysed by three cycles of freezing and thawing. Cell debris was removed by centrifugation at 12,000X g for 20 min. Packaged
5 rAAV was purified from adenovirus by two rounds of cesium chloride equilibrium density gradient centrifugation. Residual adenovirus contamination was inactivated by heating at 56°C for 45 min. Though three plasmids were used in the production of rAAV vector in this example, it is possible to combine the rAAV vector construct and the AAV helper gene construct on one plasmid. This would allow rAAV to be produced by transfecting 293
10 cells with two plasmids. Alternatively, one could add the adenovirus helper genes to this plasmid to make a single plasmid containing all that is required to make rAAV particles.

To estimate total number of rAAV particles, the virus stock was treated with DNase I, and encapsidated DNA was extracted with phenol-chloroform, and precipitated with ethanol. DNA dot blot analysis against a known standard was used to determine titer
15 (*Blood* 76:1997-2000, 1990).

To assay for adenovirus contamination, 293 cells were infected with 10 µl of purified rAAV stock and followed for any signs of cytopathic effect. All stocks were negative for adenovirus contamination (level of detection greater than or equal to 100 PFU/ml).

20 To assay for wild type AAV, 293 cells were co-infected with serial dilutions of rAAV stocks and adenovirus dl312 at a MOI of 2. Three days later the cells were harvested, lysed by three cycles of freezing/thawing, and centrifuged to remove cell debris. The supernatant was heat inactivated (56°C for 10 min) and fresh plates of 293 cells (6×10^6) were infected in the presence of adenovirus dl312 at a MOI of 2. Forty-eight hours
25 after infection, low molecular-weight DNA was isolated (*J. Mol. Biol.* 26:365-369, 1967) subjected to agarose gel electrophoresis, and transferred to a nylon membrane. The membrane was hybridized with a biotinylated oligonucleotide probe specific for the AAV capsid region. The wild type AAV titer was defined as the highest dilution of rAAV vector

stock demonstrating a positive hybridization signal. The rAAV preparations contained less than 1 wild type AAV genome per 10^9 rAAV genomes.

EXAMPLE 2

INFECTION OF CELLS WITH RAAV-CMV-FGF-2 RESULTS IN THE EXPRESSION OF FGF-2

5

293 cells were plated the day before infection at 5×10^5 cells/well in 6-well plates and were infected with rAAV-CMV-bFGF-2 virus, prepared as described above in Example 1, at different multiplicities of infection (MOI) with and without etoposide ($3\mu\text{M}$). Etoposide is a topoisomerase inhibitor which has been shown to increase transduction efficiency of rAAV vectors (*Proc. Natl. Acad. Sci. USA*, 92:5719-5723, 1995). Forty-eight hours after infection, culture supernatant was collected and cells were lysed in 0.5 mL 1x lysis buffer (100 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 0.5% NP40, and 0.5% deoxycholate). FGF-2 in the supernatants and lysates was assayed by ELISA (cat. # DFB00, R & D Systems, Minneapolis, MN) following manufacturer's instructions. The results are shown below in Table 1.

TABLE 1

FGF-2 PRODUCTION IN 293 CELLS FOLLOWING INFECTION WITH RAAV-FGF-2

Culture Medium Supernatant (1.5 ml)

sample	infection MOI	Etoposide	FGF2 protein
1	0	-	<5 pg/ml
2	2×10^5	-	<5 pg/ml
3	2×10^4	-	<5 pg/ml
4	2×10^3	-	<5 pg/ml
1	0	+	<5 pg/ml
2	2×10^5	+	106 pg/ml \approx 300pg/24h/ 10^6 cells
3	2×10^4	+	<5 pg/ml
4	2×10^3	+	<5 pg/ml

Cell Lysate (0.5 ml)

sample	infection MOI	Etoposide	FGF2 protein
1	0	-	8.95 ng/ml
2	2×10^5	-	114. ng/ml
3	2×10^4	-	18.8 ng/ml
4	2×10^3	-	11.3 ng/ml
1	0	+	5.05 ng/ml
2	2×10^5	+	296. ng/ml \approx 300ng/24h/ 10^6 cells
3	2×10^4	+	48.0 ng/ml
4	2×10^3	+	13.2 ng/ml

EXAMPLE 3

CONSTRUCTION OF RAAV VECTORS

A. Construction of pD10-bFGF-2

5 The pD10 AAV vector is constructed by replacing the AAV gene encoding sequences of pD-10 (see Wang, X. et al. *J. Virol.* 71:3077 (1997), with the CMV promoter, multiple cloning site, and BGH polyadenylation sequences from pKm201CMV. Briefly, oligonucleotides 5'-ggattttaa acttgccggcc gcggaatttc gactctaggc c-3' (SEQ I.D. No. ____)

10 and 5'-gctgcccgagg acttgctagc tggatgatcc tccagcgagg ggatctcatg -3' (SEQ I.D. No. ____)

used to amplify the CMV expression cassette from pKm201CMV. The product of this PCR amplification is digested with SmaI and DraI and cloned into pD-10 digested with EcoRV. This new vector is named pD10-CMV.

To construct pD10-bFGF-2, the synthetic gene for bovine FGF-2 (see US Patent 5,464,774 for sequence) is digested with EcoRI and Sall, treated with T4 polymerase

15 to blunt the ends, and then cloned into the StuI site of pD10-CMV. The synthetic gene described above encodes the mature, processed form of bovine FGF-2.

B. Construction of a rAAV Vector Expressing FGF-2-Sc.

pD10-K-FGF-2-Sc (see Figure 31) was constructed by cloning the FGF-2 full length humanized bovine cDNA obtained from Scios, into the pD10 vector backbone

containing the kanamycin (Kan) resistance gene. This cDNA contains the mutations at amino acid positions 121 and 137 described in example 1. Briefly, the FGF-2-Sc cDNA was digested from the parent plasmid with the enzyme NdeI, the ends blunted with T4 DNA polymerase, cut with the restriction enzyme HindIII, and cloned into the pD10-CMV vector which had been digested with the enzymes StuI and HindIII. The nucleotide sequence of pD10-K-FGF-2-Sc is illustrated in Figure 32.

C. Infection of Cells with rAAV-FGF-2-Sc Results in the Expression of FGF-2

293 cells were infected as in example 2, with the following modifications: 4 x 10⁵ cells/well were plated in a 12 well dish, and all wells contained 3 uM etoposide. Three different particle numbers of FGF-2 virus, and a negative control CMV-lacZ virus were used to infect the cells. 48 hours after infection, tissue culture media was collected and cells lysed in 100 ul lysis buffer containing Triton-X 100 and Complete™ protein inhibitor cocktail (Boehringer Mannheim, Germany). FGF-2 protein in the media and lysates was assayed by ELISA. The results are shown below in Table 2 below.

15

TABLE 2.
FGF-2 PRODUCTION IN 293 CELLS FOLLOWING INFECTION WITH RAAV
D10-K-FGF-2-Sc

Vector	Viral Particles	Lysates(pg/mL)	Culture media (pg/mL)
D10-K-FGF-2-Sc	1 x 10 ¹⁰	324492.919	438.621
D10-K-FGF-2-Sc	1 x 10 ⁹	32876.106	46.984
D10-K-FGF-2-Sc	1 x 10 ⁸	6950.039	14.649
CMV -lacZ	1 x 10 ¹⁰	2327.527	17.096

EXAMPLE 4

CONSTRUCTION OF RAAV VECTORS WHICH EXPRESS FGF5 AND FGF18

A. Cloning FGF-5 into the pD10-CMV rAAV Vector.

The FGF-5 coding region (see U.S. Serial No. 08,602,147) was cloned into
5 the rAAV pD10-CMV vector by digestion with the enzymes SacII and XmnI, resulting in
an 814 bp fragment. This removed an ORF (ORF-1) upstream of and overlapping with the
FGF-5 coding region. The ends of the FGF-5 fragment were then blunted with T4 DNA
polymerase, and it was cloned into the rAAV pD10-CMV vector linearized with StuI. This
vector contains a 1353 bp insertion of a bacteriophage Phi X174 HaeIII fragment.

10 The pD10-CMV-FGF-5 vector is illustrated schematically in Figure 3. In
summary, this plasmid contains the CMV immediate/early enhancer + promoter, the CMV
intron A, an FGF-5 coding region, the bovine growth hormone polyA site, and AAV ITR
sequences. There is a 1353 bp insertion of PhiX 174 bacteriophage DNA cloned into the
NotI site between one ITR and the CMV immediate early enhancer + promoter region.

15 B. Packaging and Functional Analysis of FGF-5 rAAV.

rAAV virus was packaged using a triple transfection method as described in
Example 1. However, rather than cesium chloride equilibrium density gradient
centrifugation, heparin sulfate column chromatography is utilized. More specifically, a cell
pellet is resuspended in TNM buffer: 20mM Tris pH 8.0, 150mM NaCl, 2mM MgCl₂.
20 Deoxycholic Acid is added to 0.5% to lyse the cells. 50 U/ml Benzonase is added and the
lysed cells are incubated at 37 degrees to digest any nucleic acids. The cell debris is
pelleted and the supernatant is filtered through a 0.45um filter and then a 0.22um filter.
The virus is then loaded onto a 1.5ml Heparin sulfate column using the Biocad HPLC. The
column is then washed with 20mM Tris pH 8.0, 100mM NaCl. The rAAV particles are
25 eluted with a gradient formed with increasing concentrations of NaCl. The fractions under
the peak are pooled and filtered through a 0.22um filter before overnight precipitation with

8% PEG 8000. CaCl_2 is added to 25mM and the purified particles are pelleted and then resuspended in HBS#2: 150mM NaCl, 50mM Hepes pH7.4.

Briefly, 8×10^8 or 8×10^9 particles of the resulting FGF-5 virus were used to infect 293 cells, which were simultaneously treated with 3 μM etoposide to enhance viral expression levels. At 24 hours post-infection, tissue culture media and cell lysates were harvested and analysed by Western blotting. Briefly, protein samples were run on a 4-20% tris-glycine gradient gel, and transferred to nitrocellulose by standard procedures. After blocking with 5% milk in PBS, the membrane was incubated with an anti-human FGF-5 antibody (R and D systems, made in goat) at a dilution of 1:1,000 for one hour at room temperature. After the membrane was washed 3 times in PBS + 0.05% Tween-20, it was incubated with an anti-goat secondary antibody conjugated to peroxidase (1:5,000 dilution). The membrane was then washed and the FGF-5 protein detected by chemiluminescence.

Results of the Western blot are shown in Figure 4. Briefly, lane 1 represents 50 ng of the 29.5 Kd recombinant FGF-5 protein (R and D systems). Lane 2, media from cells infected with 8×10^9 viral particles and treated with etoposide, shows no FGF-5 expression. Lane 3 is an uninfected cell lysate control. Lane 4 and 5 are lysates from cells infected with 8×10^8 or 8×10^9 viral particles, respectively, and Lanes 6 and 7 are lysates from cells infected with 8×10^8 or 8×10^9 viral particles and treated with 3 μM etoposide. Lanes 4-7 all show positive FGF-5 expression. Lane 8 is a negative control of lysate from uninfected cells.

In summary, although the FGF-5 signal sequence was intact, FGF-5 protein was detected in the cell lysate only.

C. Cloning FGF-5 Lacking a Signal Sequence, into rAAV pD10-CMV.

Oncogenic activity is associated with the wild-type FGF-5 molecule (Zhan et al., 1988; Bates et al., 1991). To improve its safety, the codons for the first 21 amino acids of FGF-5's signal sequence were removed by PCR amplification of the above pD10-CMV-FGF-5 plasmid with the following primers: AGA/TAT/AAG/CTT/AC

C/ATG/GGT/GAA/AAG/CG T/CTC/GCC/CCC/AAA (5', 5FGFMUTB; SEQ I.D. No.) and CGC/GCG/CTC/GAG/AC C/ATG/AGG/AAT/ATT/AT C/CAA/AGC/GAA/ACT (3', 3FGF5WT; SEQ I.D. No. ____). The 5' primer contains point mutations which destabilize G/C rich hairpin structures of the FGF-5 mRNA, and should increase levels of gene expression. The PCR product was digested with HindIII and XhoI (restriction sites introduced by the primers), and cloned by standard methods, into the pD10 vector digested with the same enzymes. The pD10-CMV-FGF-5 (sig-) vector is illustrated schematically in Figure 5.

In summary, the pD10-CMV-FGF-5 (sig-) plasmid contains the CMV immediate/early enhancer + promoter, the CMV intron A, the FGF-5 coding region with the modifications described in Example C above, the bovine growth hormone polyA site, and the AAV ITR sequences. There is a 1353 bp insertion of PhiX 174 bacteriophage DNA clones into the NotI site between one ITR and the CMV immediate early enhancer + promoter region.

15 D. Western Analysis of 293 Cells Transfected with pD10-CMV-FGF-5 (sig-).

Expression of FGF-5 protein was demonstrated by transient transfection of 293 cells with the plasmid pD10-CMV-FGF-5 (sig-), by standard methods. After 48 hours, tissue culture media and cell lysates were harvested. Western analysis was performed with an anti-human FGF-5 antibody (R and D systems) as described above.

20 Results of the Western analysis are provided below in Figure 6. Briefly, lane 1 represents 50 ng of the 29.5 Kd recombinant FGF-5 protein (R and D systems). Lanes 2 and 3, showing FGF-5 expression, are cell lysates from 293 cells transfected with two different clones of the pD10-CMV-FGF-5 sig- plasmid. Lane 4 is lysate from cells transfected with a negative control plasmid CMV-Epo. Lanes 5, 6 and 7 represent media from cells transfected with different clones of the pD10-CMV-FGF-5 sig- plasmid, 25 respectively, and the CMV-Epo plasmid. As is evident from this figure, FGF-5 protein was detected in the cell lysate only.

E. Generation of FGF-5 (signal -) rAAV.

FGF-5 (sig-) rAAV virus is packaged using the triple transfection method described in more detail above.

F. Cloning FGF-18 into the pD10-CMV rAAV Vector.

5 The FGF-18 coding region (see U.S. Provisional Application Serial No. 60/083,553) was cloned into the pD10-CMV vector as a PstI to EcoRV fragment, using restriction sites found in both the FGF-18 and the multiple cloning site of the pD10-CMV vector. The vector contains a 1353 bp insertion of PhiX174 bacteriophage DNA (see Example A).

10 A schematic illustration of pD10-CMV-FGF-18 is provided in Figure 7. Briefly, this plasmid contains the CMV immediate/early enhancer + promoter, the CMV intron A, the FGF-18 coding region, the bovine growth hormone polyA site, and the AAV ITR sequences. There is a 1353 bp insertion of PhiX 174 bacteriophage DNA cloned into the NotI site between one ITR and the CMV immediate early enhancer + promoter region.

15 G. Analysis of 293 Cells Transfected with pD10-CMV-FGF-18 Plasmid.

Expression of FGF-18 protein was assessed by transient transfection of 293 cells followed by Western analysis, using standard methods. Cell lysates and tissue culture media were harvested at 48 hours post transfection. An anti-peptide FGF-18 rabbit polyclonal antibody, generated against a selected polypeptide from recombinant FGF-18, 20 was used at a dilution of 1:2,500 for one hour at room temperature. The secondary antibody, an anti-rabbit IgG conjugated to peroxidase, was used at a dilution of 1:25,000.

Results of the Western analysis are provided in Figure 8. Briefly, lanes 1-3 represent 1, 2 and 10 ul of tissue culture media from cells transfected with the pD10-CMV-FGF-18 plasmid. Lane 4 is blank. Lanes 5, 6 and 7 contain 2, 10 and 20 ul of lysate from 25 the transfected cells. Lanes 8 and 9 are negative controls; 20 ul of tissue culture media and cell lysate, respectively, from uninfected cells. Lane 10 contains a positive control; an FGF-18-maltose binding protein fusion ((MBP); predicted size = 80 Kd, larger than the FGF-18 protein).

H. Packaging of the pD10CMV-FGF-18 plasmid into rAAV particles

FGF-18 rAAV virus was generated by the triple transfection method.

EXAMPLE 5

AAV – LACZ INJECTED RETINA

5 A. Subretinal injection of rAAV

Albino Sprague-Dawley rats were injected at 14 –15 days postnatal (P14-P15). Animals were anesthetized by ketamine/xylazine injection, and a local anesthetic (proparacain HCl) was applied topically to the cornea. An aperture was made through the inferior cornea of the eye with a 28 gauge needle. Subretinal injections of 2-3µl of AAV-
10 CMV-Lac-Z were then made by inserting a blunt 32 gauge needle through the opening and delivering the rAAV suspension into the subretinal space in the posterior retina. The contralateral eye was either uninjected, injected subretinally with PBS, or with a control rAAV containing a reporter gene.

B. Staining Protocol

15 Cryosections of the retina were stained with Bluo-gal for b-galactosidase reaction product of lacZ. In all wild type rats tested (3), positive staining was visible in the interior of the whole eyecup upon gross examination (see Figure 9). 100µm thick agarose or 20µm thick cryosections of retinas indicated that most of the b-gal positive staining localized to the photoreceptors. There were a small number of LacZ positive retinal
20 ganglion cells observed.

C. Anti-b-galactosidase immunocytochemistry

Sections from 3 wildtype and 2 transgenic rats were stained with a polyclonal antibody against b-galactosidase. These results were comparable to the bluo-gal results, primarily demonstrating photoreceptor-specific staining. Two out of five rats
25 showed no positive staining.

D. Results

Subretinal injection of 2 μ l of AAV-CMV-lacZ effectively transduced a large number of photoreceptor and retinal pigment epithelial (rpe) cells following a single intraocular inoculation of AAV-CMV-lacZ into the subretinal space (SRS) of the rat eye.

5 The lateral extent of lacZ reporter gene expression was typically 1/3 to 1/2 of the retinal expanse following a single AAV-CMV-LacZ injection. This finding was confirmed by blue-gal staining of the b-galactosidase reaction product of the lacZ gene as well as by immunocytochemistry using an antibody specific for b-galactosidase. The AAV-CMV-lacZ vector was effective at transducing photoreceptor and RPE cells in both the normal

10 (wildtype) and affected, degenerating (transgenic) rat retina.

EXAMPLE 6

RETINAL TISSUE ANALYSIS OF RAAV-FGF-2 INFECTED CELLS, VS. CONTROLS

A. Subretinal injection of rAAV

Line 3 albino transgenic rats (P23H-3) on an albino Sprague-Dawley

15 background (produced by Chrysalis DNX Transgenic Sciences, Princeton, NJ) were injected at the ages of P14 or P15. Animals were anesthetized by ketamine/xylazine injection, and a local anesthetic (proparacain HCl) was applied topically to the cornea. An aperture was made through the inferior cornea of the eye with a 28 gauge needle. The subretinal injections of 2 μ l were then made by inserting a blunt 32 gauge needle through

20 the opening and delivering the rAAV suspension into the subretinal space in the posterior retina. The intent was to inject into the subretinal space of the posterior superior hemisphere, but sometimes upon histological examination it was found that the injection site was located just inferior to the optic nerve head. The opposite eye was either uninjected, injected subretinally with PBS, with control rAAV containing no neurotrophin

25 or containing proteins not known to possess neurotrophic properties.

B. Histopathology Protocol / Retinal tissue analysis

The rats were euthanized by overdose of carbon dioxide inhalation and immediately perfused intracardially with a mixture of mixed aldehydes (2% formaldehyde and 2.5 % glutaraldehyde). Eyes were removed and embedded in epoxy resin, and 1 μ m thick histological sections were made along the vertical meridian. Tissue sections were aligned so that the ROS and Müller cell processes crossing the inner plexiform layer were continuous throughout the plane of section to assure that the sections were not oblique, and the thickness of the ONL and lengths of RIS and ROS were measured as described (see e.g., LaVail, et al. PNAS 1992, 89(23):11249-53 and LaVail et al., Invest. Ophthalmol. Vis. Sci 1998, 39(3):592-602). Briefly, 54 measurements of each layer or structure were made at set points around the entire retinal section. These data were either averaged to provide a single value for the retina, or plotted as a distribution of thickness or length across the retina. The 3 greatest contiguous values for ONL thickness was also compared in each retina, to determine if any region of retina (e.g., nearest the injection site) showed proportionally greater rescue; although most of these values were slightly greater than the overall mean of all 54 values, they were no different from control values than the overall mean. Thus, the overall mean was used in the data cited, since it was based on a much larger number of measurements.

C. Results

Two surgical methods of delivery of rAAV-CMV-FGF2 were completed, intravitreal and subretinal injection.

1. Intravitreal injection

RAAV-CMV-FGF-2 was injected into the right eye of nine transgenic S334ter rats after day P15 (the left eye was not injected). S334(4) transgenic animals were used to assess the rescue effect of rAAV-CMV-FGF-2 on degenerating photoreceptor cells when delivered by intravitreal injection. The rats were all sacrificed at age p60 and the embedded in plastic and sectioned to assess morphology and therapeutic effect as assayed

by the preservation of thickness of outer nuclear layer. Superior and inferior regions of eyecup are quantitated by measuring the ONL thickness using a BioQuant morphometric measuring system (BioQuant). Injected eyes were evaluated along with uninjected control eyes.

5

Control Left superior – 16.52 +/- 2.77 um

Injected Right superior – 19.71 +/- 5.27 um

Control Left inferior – 22.64 +/- 2.11 um

10

Injected Right inferior – 26.47 +/- 3.55 um

Based upon these results it was evident that there is a rescue effect of AAV-CMV-FGF-2 when delivered intraocularly into the vitreous cavity.

2. Subretinal injection of rAAV-CMV-FGF-2

15

Experiment A. - Location of injection – subretinal , 7 rats-both right and left eyes injected, 3 rats-(left eye = uninjected). Number of rats injected – 10 rats all wild type p15 on day of injection. One rat was sacrificed every week starting at week 2. Expression of FGF-2 was assessed, as well as any signs of inflammation or neovascularization.

Experiment B. - Location of injection – subretinal, 5 rats- right eyes injected
20 w/vector left eyes injected with PBS, 4 rats- right eyes injected w/vector (left eye = uninjected). Number of rats injected – 11 transgenic S334(4) rats – all were p15 on day of injection. The rats were sacrificed at age p60 and the embedded in plastic and sectioned to assess histopathology and number of surviving photoreceptor cells. .

Anatomic indication of therapeutic effect (photoreceptor rescue) was
25 assessed histologically. Briefly, eyes injected with rAAV-CMV-FGF2 retained significantly more photoreceptors at P60, P75 and P90 than uninjected contralateral control eyes of the same animal. Retinas receiving a subretinal injection of AAV-CMV-FGF2 at

P14-15 retained 71% of the normal ONL thickness, compared to about 47% in the uninjected controls (see Figures 11, 12, 13 and 14).

There was little or no rescue in PBS-injected control eyes ($p > 0.169$ in all cases). This is consistent with previous reports that needle injury to the retina in young rats (P14-P15) does not rescue photoreceptors or up-regulate bFGF mRNA expression.

3. Subretinal injection of rAAV-CMV-FGF-2

Two to three microliters of rAAV-CMV-FGF-2 vector was injected into the subretinal space between the photoreceptors and the adjacent retinal pigment epithelium at P14 or P15. Rats were sacrificed and eyes examined at time points between P60-P90. At these ages in uninjected control eyes of S334ter rats, the ONL thickness, which is an index of photoreceptor cell number, was reduced to about 60% of normal.

Evidence of anatomic rescue was found to be significant to the $p = .005$ confidence level in retinas transfected by rAAV-CMV-FGF-2 when compared to the control AAV vectors or sham injection of PBS by ANOVA (analysis of Variance statistical measures). JMP statistical analysis software (Copyright (c) 1999 SAS Institute Inc. Cary, North Carolina, USA).

EXAMPLE 7

ANTIBODY STAINING OF RAAV-FGF-2 INFECTED CELLS

A. Injection Protocol

Albino Sprague-Dawley rats were injected with rAAV-CMV-FGF-2 at the ages of P14 or P15 essentially as follows. Briefly, wild-type animals were anesthetized by ketamine/xylazine injection, and a local anesthetic (proparacain HCl) was applied topically to the cornea. An aperture was made through the inferior cornea of the eye with a 28 gauge needle. The subretinal injections of 2-3 ul of rAAV-CMV-FGF-2 were then made by inserting a blunt 32 gauge needle through the opening and delivering the rAAV suspension

into the subretinal space in the posterior retina. The contralateral eye was either uninjected, injected subretinally with PBS, wild-type rAAV, or with rAAV-CMV-lacZ.

B. Staining Protocol

Fixed eyecups were embedded in OCT and cryosectioned in 20um thick
5 sections. Sections from 10 wt-rats were stained with antibody to FGF-2. (primary- anti-FGF-2 1:500 (commercial antibody purchased from R&D systems) (secondary-anti-goat Cy3 conjugate (Sigma, St. Louis. MO)

C. Results

Immunohistochemistry was used to look for expression of FGF-2 in the eye.
10 Two rats were examined every week starting at 3 weeks post-injection. Retinas were examined for expression of FGF-2 and also examined histopathologically for signs of inflammation or neovascularization.

Results are shown in Figure 15. Briefly, expression of FGF-2 was found in retinal photoreceptor cells as well as RPE cells at 35 days following inoculation with 2-3 ul
15 of rAAV-CMV-FGF-2. Less significant expression was noted in retinal bipolar interneurons and retinal ganglion cells (RGCs) following injection into the subretinal space (SRS). No significant staining above background was observed in sections injected with PBS or rAAV-CMV-lacZ vectors.

EXAMPLE 8

20 RETINAL TISSUE ANALYSIS OF RAAV-FGF-5 (SIG-) AND -18 INFECTED CELLS, VS. CONTROLS

A. Subretinal injection of rAAV

Line 4 albino transgenic rats (S334ter-4) on an albino Sprague-Dawley background (produced by Chrysalis DNX Transgenic Sciences, Princeton, NJ) were injected at age P15. Animals were anesthetized by ketamine/xylazine injection, and a local
25 anesthetic (proparacain HCl) was applied topically to the cornea. An aperture was made through the inferior cornea of the eye with a 28 gauge needle. The subretinal injections of

2.5 μ l were then made by inserting a blunt 32 gauge needle through the opening and delivering the rAAV suspension into the subretinal space in the posterior retina. The opposite eye was either uninjected, injected subretinally with PBS, with control rAAV containing no neurotrophin or containing proteins not known to possess neurotrophic properties.

B. Histopathology Protocol / Retinal tissue analysis

The rats were euthanized by overdose of carbon dioxide inhalation and immediately perfused intracardially with a mixture of mixed aldehydes (2% formaldehyde and 2.5 % glutaraldehyde). Eyes were removed and embedded in epoxy resin, and 1 μ m thick histological sections were made along the vertical meridian. Tissue sections were aligned so that the ROS and Müller cell processes crossing the inner plexiform layer were continuous throughout the plane of section to assure that the sections were not oblique, and the thickness of the ONL was measured as described (LaVail, et al). Briefly, 54 measurements of each layer or structure were made at set points around the entire retinal section. The 27 measurements from the inferior region and the superior region of the retina were averaged separately to give two values for each eye. This separation was made because the retina degenerates at different rates in these two regions of the S334ter-4 animal model.

C. Results

Sub-retinal injections of both rAAV-CMV-FGF-5 (sig-) and rAAV-CMV-FGF-18 were performed.

1. Sub-retinal injection of rAAV-CMV-FGF-5 (sig-)

Experiment. - Location of injection – subretinal, 3 rats- right eyes injected w/vector left eyes injected with PBS, 8 rats- right eyes injected w/vector left eyes injected with rAAV-CMV-LacZ, 4 rats- right eyes injected w/vector (left eye = uninjected). Number of rats injected – 15 transgenic S334ter-4 rats – all were p15 on day of injection.

The rats were sacrificed at age p60. The retinas were embedded in plastic and sectioned to assess histopathology and number of surviving photoreceptor cells.

Anatomic indication of therapeutic effect (photoreceptor rescue) was assessed histologically. The injection of rAAV-CMV-FGF-5 (sig-) resulted in significant rescue of photoreceptors, compared to PBS injected, rAAV-CMV-LacZ injected, and uninjected eyes (see Figure 33). The rescue was significant to the $p=0.05$ confidence level for all three comparisons, by ANOVA (analysis of Variance statistical measures). JMP statistical analysis software (Copyright (c) 1999 SAS Institute Inc. Cary, North Carolina, USA).

10 2. Sub-retinal injection of rAAV-CMV-FGF-18

Experiment. - Location of injection – subretinal, 3 rats- right eyes injected w/vector left eyes injected with PBS, 3 rats- right eyes injected w/vector left eyes injected with rAAV-CMV-LacZ, 4 rats- right eyes injected w/vector (left eye = uninjected). Number of rats injected – 10 transgenic S334ter-4 rats – all were p15 on day of injection.

15 The rats were sacrificed at p60, and the retinas embedded in plastic and sectioned.

Eyes injected with rAAV-CMV-FGF-18 retained significantly more photoreceptors at P60 than PBS injected, rAAV-CMV-LacZ injected, or uninjected control eyes. Each comparison, by ANOVA, was statistically significant to the $p=0.05$ confidence level.

20 EXAMPLE 9

IN VIVO DELIVERY TO RETINAL GANGLION CELLS (RGCs)

A. Intra-vitreous injection of AAV vectors

All surgical procedures were performed in female adult Sprague-Dawley rats (180-200 g; Charles River Breeders) under general anesthesia (7% chloral hydrate; 0.42 mg per g of body weight, i.p.) in accordance with the Use of Animals in Neuroscience

Research and McGill University Animal Care Committee guidelines for the use of experimental animals.

Briefly, rAAV-CMV-lacZ (5 μ l; see above) was injected into the vitreous chamber in the superior (dorsal) hemisphere of the retina using a posterior approach as described (Di Polo, PNAS, 1998). Control eyes were injected with equal volumes of
5 Hepes-buffered saline (HBS, virus vector).

B. Identification of RPCs

For visualization of RGCs, neurons were retrogradely labeled with the fluorescent tracer Fluorogold (Fluorochrome, Englewood, CO) at 2% in 0.9% NaCl
10 containing 10% dimethyl sulfoxide by application of the tracer to both superior colliculi 7 days prior to analyses as described (Vidal-Sanz *et al.*, 1988). Anesthetized rats were then perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and the eyes were immediately enucleated. The anterior part of the eye and the lens were removed and the remaining eye cup was immersed in the same fixative for 2 hr at 4°C. Eye
15 cups were cryoprotected in graded sucrose solutions (10-30% in PB) for several hours at 4°C, embedded in O.C.T. compound (Tissue-Tek, Miles Laboratories, Elkhart, IN) and frozen in a 2-methylbutane/liquid nitrogen bath. Retinal radial cryosections (12-15 μ m), obtained along the vertical meridian of the eye, were collected onto gelatin-coated slides and processed for immunocytochemistry. Alternatively, entire eyes were rinsed three times
20 (15 min each) in PBS at room temperature with gentle shaking, to whole-mount histochemical staining as described below.

C. Histochemical Analysis

Expression of the bacterial LacZ gene in whole retinas was detected by standard histochemical staining reactions using halogenated indoyl- β -D-galactoside
25 (Bluogal; GIBCO BRL). Following removal of the anterior eye structures and lens, eye cups were incubated overnight in a staining solution containing 5mM K-ferricyanide, 5

mM K-ferrocyanide, 2 mM MgCl₂, and 0.5 mg/ml Bluo-gal at 37°C. Retinas were then dissected, fixed for an additional 30 min and flat-mounted vitreal side up on glass slides.

For visualization of the AAV-mediated lacZ gene product in retinal radial sections, cryosections were incubated in 10% normal goat serum (NGS) in 0.2% Triton X-100 (Sigma, St. Louis, MO) in phosphate buffer saline (PBS) for 30 min at room temperature to block non-specific binding. Two primary antibodies raised against the lacZ gene product were used with similar results. A polyclonal anti-beta-gal antibody (diluted 1:1000; 5 prime→3 prime, Inc., Boulder, CO) and a monoclonal anti-LacZ antibody (diluted 1:500; Promega, Madison, WI). Primary antibodies were added in 2% NGS in 0.2% Triton X-100 and incubated overnight at 4°C. Sections were subsequently processed with anti-rabbit Cy3-conjugated IgG (diluted 1:500, Jackson ImmunoResearch, West Grove, PA) or anti-mouse Cy3-conjugated IgG (diluted 1:500, Jackson ImmunoResearch) and mounted. Control sections were treated in the same way but with omission of the primary antibody. Sections were visualized by fluorescent microscopy (Polyvar, Reichert-Jung).

Expression of the heparan sulfate proteoglycan receptor in the retina was examined using a monoclonal anti-heparan sulfate antibody (HepSS-1, diluted 1:1,000, Seikagaku Corporation, Tokyo, Japan). Following overnight incubation at 4°C, sections were processed with biotinylated anti-mouse Fab fragment (Jackson ImmunoResearch), avidin-biotin-peroxidase reagent (ABC Elite Vector Labs, Burlingame, CA), followed by reaction in a solution containing 0.05% diaminobenzidine tetrahydrochloride (DAB) and 0.06% hydrogen peroxide in PB (pH 7.4) for 5 min. For analysis of co-localization of heparan sulfate in Fluorogold-labeled neurons, sections were processed with Cy3-coupled anti-mouse IgG (Jackson ImmunoResearch) after incubation in primary antibody. In all cases, the primary antibody was omitted in control sections. Sections were mounted and visualized by light or fluorescent microscopy.

Quantification of AAV-transduced cells in the ganglion cell layer of retinal flat-mounts was performed in two ways: i) by counting the entire number of Bluo-gal positive cells in each of the retinal quadrants (superior, inferior, temporal and nasal); and ii)

by counting the number of cells in three standard areas (at 1, 2 and 3 mm from the optic disc) of each quadrant as previously described (Villegas-Perez *et al.*, 1993).

For quantification of Fluorogold, Blue-gal or HepSS-1 positive cells in retinal radial sections, the entire number of labeled cells per section was counted under
5 fluorescent microscopy. Four to five serial sections per eye were routinely counted and a mean value per animal was obtained, followed by the calculation of a mean value for the entire experimental group which consisted of 4-5 rats. Results were analyzed using the Sigmastat program (Jandel, San Rafael Madera, CA) by a student's *t* test (paired groups).

D. Results

10 Analysis of retinas from eyes that received a single intravitreal injection of rAAV-CMV-lacZ demonstrated a large number of LacZ-positive cells throughout the entire GCL as assessed by histochemical LacZ staining of both flat-mounts (Figure 16A) and radial sections (Figure 16B). In many cases, RGCs transduced by AAV could be unequivocally identified because the LacZ reaction product filled their axons that
15 converged at the level of the optic nerve head. In addition, LacZ-positive photoreceptor nuclei were observed but were always restricted to the vicinity of the injection site (not shown). No staining was observed in control eyes injected with virus vector. No signs of cytotoxic damage or cellular immune reaction to the viral vector were observed in any of the retinas examined.

20 Quantification of LacZ-positive cells in the GCL of retinal flat-mounts demonstrated a 2.8-fold increase between 2 and 4 weeks after intravitreal injection of the rAAV-CMV-lacZ vector (Figure 17). For example, it was found that $27,569 \pm 7,646$ cells/retina (mean \pm S.D.; $n=3$) and $79,043 \pm 10,321$ cells/retina ($n=4$) expressed the LacZ gene product at 2 and 4 weeks after intraocular administration of the vector, respectively.
25 A comparable number of cells expressing the AAV-mediated transgene at 4 weeks was observed at 8 weeks ($70,221 \pm 12,500$; $n=3$) following rAAV-CMV-lacZ injection. Although the majority of LacZ-positive cells were observed in the superior hemisphere at 2 weeks after virus administration, there was robust transgene expression throughout the

entire retina by 4 and 8 weeks as assessed by quantification of Lac-Z positive cell densities in all retinal quadrants at these time points (Figure 17).

To identify the cellular localization of the AAV-mediated LacZ gene product, immunocytochemical staining of LacZ was combined with retrograde tracing of RGC bodies using Fluorogold backlabeling from the superior colliculi. Double-labeling experiments indicated that the majority of cells in the GCL expressing the LacZ gene product were also Fluorogold-positive (Figure 18). Analysis of the number of Fluorogold-labeled cells in the GCL expressing the LacZ gene product indicated that 300 ± 29 (mean \pm S.D.; $n=4$) expressed both markers out of 330 ± 32 Fluorogold-labeled cells (the average RGC population per retinal radial section). This indicates that $\sim 92\%$ of RGCs, identified by the Fluorogold label, also expressed the AAV-mediated LacZ gene product (Figure 19). In all retinas examined, it was routinely observed that a number of Lac-Z positive cells that were not labeled with Fluorogold (Figures 18 and 19). Thus, it is possible that these cells are displaced amacrine cells or RGCs that failed to incorporate the retrograde tracer. Together, these results indicate that RGCs are preferentially transduced by recombinant AAV following intravitreal injection of this viral vector.

To investigate the molecular mechanisms underlying preferential transduction of RGCs by AAV, expression of the heparan sulfate proteoglycan (which mediates both AAV attachment and infection of target cells (Summerford *et al.*, 1998)) in the adult retina was observed. Immunostaining of retinal radial sections with a specific antibody against heparan sulfate (HepSS-1) demonstrated robust staining in the GCL (Figure 20). Positive immunolabeling was clearly visualized in both neuronal somata and axonal bundles in the fiber layer. More diffuse and sparse staining was observed in some photoreceptor nuclei and cells in the inner nuclear layer. No staining was observed in control retinal sections in which the primary antibody was omitted (not shown).

To determine the cell type within the GCL that express the heparan sulfate proteoglycan receptor, a double-labeling study was performed in which RGCs were first retrogradely labeled from the superior colliculi followed by immunostaining of retinal sections with HepSS-1. Our analysis showed that 299 ± 23 (mean \pm S.D.; $n=4$) cells in the

GCL expressed both Fluorogold and HepSS-1 markers out of 315 ± 34 Fluorogold-labeled cells which represent the average RGC population visualized per retinal radial section. The large population of RGCs (~95%) expressing heparan sulfate proteoglycan receptor correlated well with the number of RGCs expressing the AAV-mediated transgene product
5 (~92%). Together, these data suggest that preferential transduction of adult RGCs by recombinant AAV is mediated by the heparan sulfate proteoglycan receptor expressed by these neurons.

EXAMPLE 10

CONSTRUCTION OF A RAAV VECTOR EXPRESSING 10 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) 165

The human VEGF-165 cDNA was cloned from the PCR-Blunt II Topo Vector (Invitrogen) into the pD10-CMV rAAV vector as an EcoR1 fragment (pD10-VEGFUC). The pD10-VEGFUC vector is illustrated schematically in Figure 21, and its
15 nucleotide sequence is shown in Figure 22. The VEGFUC rAAV virus was packaged using the triple transfection method and purified by column chromatography.

Briefly, a cell pellet is resuspended in TNM buffer: 20mM Tris pH 8.0, 150mM NaCl, 2mM $MgCl_2$. Deoxycholic Acid is added to 0.5% to lyse the cells. 50u/ml Benzonase is added and the lysed cells are incubated at 37 degrees to digest any nucleic
20 acids. The cell debris is pelleted and the supernatant is filtered through a 0.45um filter and then a 0.22um filter. The virus is then loaded onto a 1.5ml Heparin sulfate column using the Biocad. The column is then washed with 20mM Tris pH 8.0, 100mM NaCl. The rAAV particles are eluted with a gradient formed with increasing concentrations of NaCl. The fractions under the peak are pooled and filtered through a 0.22um filter before
25 overnight precipitation with 8% PEG 8000. $CaCl_2$ is added to 25mM and the purified particles are pelleted and then resuspended in HBS#2: 150mM NaCl, 50mM Hepes pH7.4.

EXAMPLE 11

INFECTION OF 293 CELLS WITH D10-VEGF RAAV RESULTS IN VEGF PROTEIN EXPRESSION

The functionality of the viral particles was assessed by infection of 293 cells with 3 different viral multiplicities of infection (MOIs); 1×10^7 , 1×10^8 and 1×10^9 viral particles per 4×10^5 293 cells, in the presence of 1.5 μ M etoposide. At 48 hours post infection, tissue culture media (supers) and cell lysates were harvested. VEGF protein levels were determined using a Quantikine human VEGF sandwich ELISA kit (R and D Systems, see Figure 23). VEGF protein concentrations are given in pg/ml. The two highest MOIs gave values significantly above that of the cells infected with a negative control virus. The levels of secreted VEGF were approximately 4-7 fold higher than those of the lysates.

EXAMPLE 12

INFECTION OF RETINAL PIGMENT EPITHELIAL (RPE) CELLS WITH D10-VEGF RAAV RESULTS IN VEGF PROTEIN SECRETION

VEGF expression levels in a monolayer of cultured primary (or very early passage) human fetal RPE cells infected with D10-VEGF 165 rAAV were clearly elevated relative to endogenous levels. Cells were infected with a range of rAAV particles from 0 to 1×10^5 per cell. VEGF expression was dose dependent, increased over time, and secretion appeared to be somewhat higher from the apical surface. In a representative experiment, RPE cells infected with 1×10^5 viral particles secreted $> 100 \text{ ng}/1 \times 10^6$ cells from the apical surface and $50 \text{ ng}/1 \times 10^6$ cells from the basal surface at 8 days post infection. The polarity of VEGF secretion from human fetal RPE cells infected with 3 different MOIs is shown in Figure 24.

EXAMPLE 13

INFECTION OF RETINAL PIGMENT EPITHELIAL (RPE) CELLS WITH VEGF RAV RESULTS IN
VEGF PROTEIN SECRETION AND DECREASED MEMBRANE CONDUCTANCE

5 Infection of cultured human fetal RPE cells with recombinant VEGF
adenovirus (AV) results in secretion of very high levels of VEGF from both the apical and
basal surfaces of the RPE. MOI's of 0 to 1,000 or 0 to 10,000 particles per cell were used
in two separate experiments. In both cases, expression levels increased over time, peaking
at approximately 100-200 ug/1 x 10⁶ cells at the highest MOI's (see Figure 25). In
10 addition, the total transepithelial membrane resistance of the RPE monolayer decreased
significantly at all MOIs, and by approximately 4-5 fold at the highest MOIs (see Figure
26).

EXAMPLE 14

CONSTRUCTION OF A RAAV VECTOR EXPRESSING SOLUBLE FLT-1 (SFLT-1) RECEPTOR

15 The sFlt-1 cDNA was cloned from the Blunt II Topo Vector (Invitrogen)
into the pD10-CMV rAAV vector as an EcoR1 fragment (pD10-sFlt-1). The pD10-sFlt-1
vector is illustrated schematically in Figure 27, and its nucleotide sequence is shown in
Figure 28. The human sFlt-1 rAAV virus was packaged using the triple transfection
20 technique and purified by column chromatography.

EXAMPLE 15

IN VITRO ASSAY FOR ANTI-ANGIOGENIC ACTIVITY

25 This example describes the HMVEC (human dermal microvascular
endothelial cell) proliferation assay, which can be utilized to determine the anti-angiogenic
activity of a molecule by inhibition of VEGF stimulated proliferation (see Kupprion et al.,
1998. JBC 273:29635-29640).

Briefly, HMVEC cells obtained from Clonetics (catalog #2543), are seeded in a 96 well culture dish at a density of 2,000 cells per well in 100 ul assay media and incubated at 37°C for 3-5 hours. The assay media is EBM media, or endothelial basal media (Clonetics, catalog # CC-3121) containing 5% FBS and 1% pen/strep. Dilutions of
5 anti-angiogenic samples are added in triplicate (50 ul each, for final well volume of 200 ul), immediately followed by 50 ul 20 ng/ml recombinant VEGF (R and D Systems, final concentration 5 ng/ml, or 0.1 nM). The samples are conditioned media from 293 cells transiently transfected with a pD10 rAAV plasmid, by standard methods, or from 293 cells infected with an rAAV virus. Culture media is collected from 24-48 hours post-
10 transfection or post-infection. After the addition of sample and recombinant VEGF, the cells are incubated for 48 hours at 37°C, when they are pulsed with 1 uCi/well ³H-thymidine (Amersham, catalog # TRK300). The stock of 1 mCi/ml ³H-thymidine is diluted 1:10 in assay media, and 10 ul added per well. The cells are incubated for an additional 18 hours, at which point 100 ul media is removed and the cells are lysed by the addition of 40
15 ul of 1M NaOH per well. Finally, the cells are harvested with a Tomtec Harvester, transferred to Filtermat paper (Wallac), 10 ml of scintillation fluid added and the incorporation of ³H-thymidine determined by scintillation counting.

Inhibition of HMVEC proliferation by conditioned media from sFlt-1 rAAV infected 293 cells is shown in Figure 34. Complete inhibition of proliferation (induced by
20 0.1 nM VEGF) was observed at approximately 1 nM sFlt-1, and partial inhibition at approximately 0.3 nM and 0.1 nM sFlt-1 protein. This inhibition was not seen with HMVEC cells treated with conditioned media from lacZ rAAV infected cells, or cells treated with recombinant VEGF alone. To generate conditioned media, 4 x 10⁵ 293 cells were infected with 1 x 10¹¹ total particles sFlt-1 rAAV or a CMV-lacZ control rAAV. If
25 one assumes an infectious particle ratio of 1:1,000 to 1:10,000, this is an MOI of less than or equal to 2.5 x 10² per cell. All samples were run in triplicate, and means and standard deviations are shown. Background incorporation of ³H-thymidine in cells not stimulated with exogenous VEGF was subtracted from all samples, leading to a negative value in one

case. The total amount of sFlt-1 protein in the conditioned media was determined by a sandwich Elisa (antibodies purchased from R and D Systems).

5

EXAMPLE 16

ANIMAL MODEL FOR NEOVASCULARIZATION BY SUBRETINAL INJECTION OF VEGF RAAV

This example describes an animal model that, after subretinal injection of a recombinant virus (rAAV) containing an angiogenic transgene (VEGF), generates
10 subretinal neovascularization and choroidal neovascularization. As noted above, choroidal neovascularization is a hallmark of exudative or wet Age-related Macular Degeneration (AMD), the leading cause of blindness in the elderly population. Retinal neovascularization occurs in diseases such as diabetic retinopathy and retinopathy of prematurity (ROP), the most common cause of blindness in the young.

15 Briefly, subretinal injections of 2 μ l AAV-VEGF (titer: 5.8×10^{13} particles/ml) were made in a bleb under the retina just outside the apical membrane of the RPE. These injections were made 3-3.5 months before the animals were sacrificed. After sacrifice animals were examined for the extent and duration of neovascularization induced by rAAV vectors using fundus photography, fluorescein angiography (Figure 35),
20 histology (Figure 36), and immunochemistry (Figure 37). As described in more detail below, these figures demonstrate in three different ways that AAV mediated overexpression of VEGF in the RPE can generate choroidal neovascularization.

More specifically, Figure 35A is an image of the fundus showing retinal blood vessels from a live animal before it was sacrificed for the serial sectioning shown in
25 Figure 36. Note that the blood vessels are larger in diameter in the AAV-VEGF injected area (black arrow). Figure 35B, is a fluorescein angiogram from the same animal taken shortly after the fundus image in 35A. Two minutes after fluorescein injection (intramuscular) significant leakage of fluorescein was observed at the AAV-VEGF injection site (black arrows).

Figure 36 (panels A-D) are a series of epoxy sections taken after sacrifice of the animal. Figure 36A is a section taken from half of the eye furthest from AAV-VEGF injection site, showing normal photoreceptors and blood vessels (the arrow points to photoreceptors with normal morphology). Moving from this section toward the AAV-VEGF injection site, monotonically increasing photoreceptor disorganization and new blood vessel formation was observed (data not shown).

Figures 36B and C are epoxy sections from the AAV-VEGF injection site. The short arrows show new blood vessel growth and the long arrows show a pathologically disorganized photoreceptor layer. Figure 36D is also an epoxy section from the AAV-VEGF injection site. The arrow points to a blood vessel breaking through Bruch's Membrane, probably from the choroid.

Figures 37(A-D) show lectin / BrdU double-staining of rat retina (A-B) and choroid (C-D). Green staining is the lectin staining of endothelial cells, and red staining is BrdU staining of dividing cells. Green cells with red dots are dividing endothelial cells, which are part of the newly formed blood vessels.

More specifically, Figure 37A is an image of the at the AAV-VEGF injection site showing extensive BrdU staining. Note that there are many more blood vessels compared to that seen at points distant from the AAV-VEGF injection site in panel B. The lectin staining is fuzzy because the blood vessels are bloated with dividing endothelial cells. Figure 37B is an image of the retina furthest from the AAV-VEGF injection site. This image shows minimal BrdU staining. In contrast, lectin staining is clear and sharply defined. Figure 37C is an image of the choroids at the AAV-VEGF injection site, which also shows extensive BrdU staining. The lectin image is not shown since it binds indiscriminately throughout choroid and sclera. Figure 37D is an image of the choroids furthest from the AAV-VEGF injection site, and shows minimal BrdU staining.

EXAMPLE 17

INJECTION OF THERAPEUTIC ANTI-ANGIOGENIC RAAV INTO AN ANIMAL MODEL FOR
OCULAR NEOVASCULARIZATION

This example demonstrates the ability of anti-angiogenic molecules to
5 prevent neovascularization in the rat model described above. Briefly, in four animals
rAAV-sFlt-1 or rAAV-PEDF were injected together with rAAV-VEGF into the subretinal
space of one eye; the contralateral eye received an injection of rAAV-VEGF and rAAV-
GFP. Six weeks after injection, electroretinographic analysis (ERGs) were obtained from
both eyes of each animals simultaneously. The a- or b- wave amplitude from the test eye
10 (AAV-VEGF+AAV-sFlt) is designated as A_t or B_t . The a- or b- wave amplitude from the
control eye (AAV-VEGF+AAV-GFP) is designated as A_c or B_c , respectively. In each eye
ERG a- and b- waves were measured and for each animal the amplitude ratio was
calculated as percent change in the test eye relative to the control eye:

15
$$\text{ERG Amplitude Ratio} = (A_t - A_c) / A_c \text{ or } (B_t - B_c) / B_c$$

The amplitude ratio for a- and b- waves are plotted in Figure 38. Briefly,
significant functional rescue was obtained in three/four animals using sFlt-1 and two/four
animals using PEDF. Figure 39 shows comparison ERGs of test and control eye of sFlt-1
20 treated rats. The dark-solid line shows that the eye rescued with sFlt-1 has a- and b- wave
amplitudes approximately twice as large as the control eye (light-dashed line). This data
indicates that sFlt-1 or PEDF can be used to rescue retinal function that is lost during
neovascularization.

25 From the foregoing, it will be appreciated that, although specific
embodiments of the invention have been described herein for purposes of illustration,
various modifications may be made without deviating from the spirit and scope of the
invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

We claim:

1. A method of treating or preventing diseases of the eye, comprising, administering intraocularly a gene delivery vector which directs the expression of a neurotrophic factor, such that said disease of the eye is treated or prevented.
2. The method according to claim 1 wherein said neurotrophic factor is NGF, BDNF, CNTF, NT-3, or, NT-4.
3. The method according to claim 1 wherein said neurotrophic factor is a FGF.
4. The method according to claim 3 wherein said FGF is FGF-2, FGF-5, FGF-18, FGF-20, or, FGF-21.
5. The method according to claim 1 wherein said disease of the eye is macular degeneration.
6. The method according to claim 1 wherein said disease of the eye is diabetic retinopathy.
7. The method according to claim 1 wherein said disease of the eye is an inherited retinal degeneration.
8. The method according to claim 7 wherein said inherited retinal degeneration is retinitis pigmentosa.

9. The method according to claim 1 wherein said disease of the eye is glaucoma.
10. The method according to claim 1 wherein said disease of the eye is a surgery-induced retinopathy.
11. The method according to claim 1 wherein said disease of the eye is retinal detachment.
12. The method according to claim 1 wherein said disease of the eye is a photic retinopathy.
13. The method according to claim 1 wherein said disease of the eye is a toxic retinopathy.
14. The method according to claim 1 wherein said disease of the eye is a trauma-induced retinopathy.
15. The method according to claim 1 wherein said gene delivery vector is a retrovirus selected from the group consisting of HIV and FIV.
16. The method according to claim 1 wherein said gene delivery vector is a recombinant adeno-associated viral vector.
17. A method of inhibiting neovascular disease of the eye, comprising, administering intraocularly a gene delivery vector which directs the expression of an anti-angiogenic factor, such that said neovascular disease of the eye is inhibited.

18. The method according to claim 17 wherein said anti-angiogenic factor is soluble Flt-1, PEDF, soluble Tie-2 receptor, or, a single chain anti-VEGF antibody.

19. The method according to claim 17 wherein said neovascular disease of the eye is diabetic retinopathy, wet AMD, and retinopathy of prematurity.

20. The method according to claim 17 wherein said gene delivery vector is a retrovirus selected from the group consisting of HIV and FIV.

21. The method according to claim 17 wherein said gene delivery vector is a recombinant adeno-associated viral vector.

22. A gene delivery vector which directs the expression of a neurotrophic factor, or an anti-angiogenic factor.

23. The gene delivery vector according to claim 22 wherein said neurotrophic factor is NGF, BDNF, CNTF, NT-3, or, NT-4.

24. The gene delivery vector according to claim 22 wherein said neurotrophic factor is a FGF.

25. The gene delivery vector according to claim 22 wherein said FGF is FGF-2, FGF-5, FGF-18, FGF-20, or, FGF-21.

26. The gene delivery vector according to claim 22 wherein said anti-angiogenic factor is soluble Flt-1, PEDF, soluble Tie-2 receptor, or, a single chain anti-VEGF antibody.

27. The gene delivery vector according to claim 22 wherein said vector is generated from a retrovirus.

28. The gene delivery vector according to claim 27 wherein said retrovirus is HIV or FIV.

29. The gene delivery vector according to claim 22 wherein said vector is generated from a recombinant adeno-associated virus.

30. A non-human animal model of neovascularization of the eye, comprising an animal having an angiogenic transgene in the eye.

31. The non-human animal model according to claim 30 wherein said neovascularization is retinal neovascularization.

32. The non-human animal model according to claim 30 wherein said neovascularization is choroidal neovascularization.

33. The non-human animal model according to claim 30 wherein said animal is a mouse or rat.

34. The non-human animal model according to claim 30 wherein said angiogenic transgene encodes VEGF.

35. The non-human animal model according to claim 30 wherein said angiogenic transgene encodes an angiopoietin.

36. A method for making a non-human animal model of neovascularization of the eye, comprising administering to a non-human animal a gene delivery vector which directs the expression of an angiogenic transgene.

37. The method according to claim 36 wherein said gene delivery vector is administered subretinally.

38. The method according to claim 36 wherein said gene delivery vector is administered intravitreally.

39. The method according to claim 36 wherein said gene delivery vector is rAV or rAAV.

40. The method according to claim 36 wherein said angiogenic transgene is a nucleic acid molecule which encodes VEGF.

41. The method according to claim 36 wherein said angiogenic transgene is a nucleic acid molecule which encodes an angiopoietin.

42. A method for determining the ability of an anti-angiogenic factor to inhibit neovascularization of the eye, comprising: (a) administering to an animal model according to any one of claims 30 to 35 an anti-angiogenic factor, and (b) determining the ability of said anti-angiogenic factor to inhibit neovascularization of the eye.

43. The method according to claim 42 wherein said anti-angiogenic factor is administered subretinally.

44. The method according to claim 42 wherein said anti-angiogenic factor is administered intravitreally.

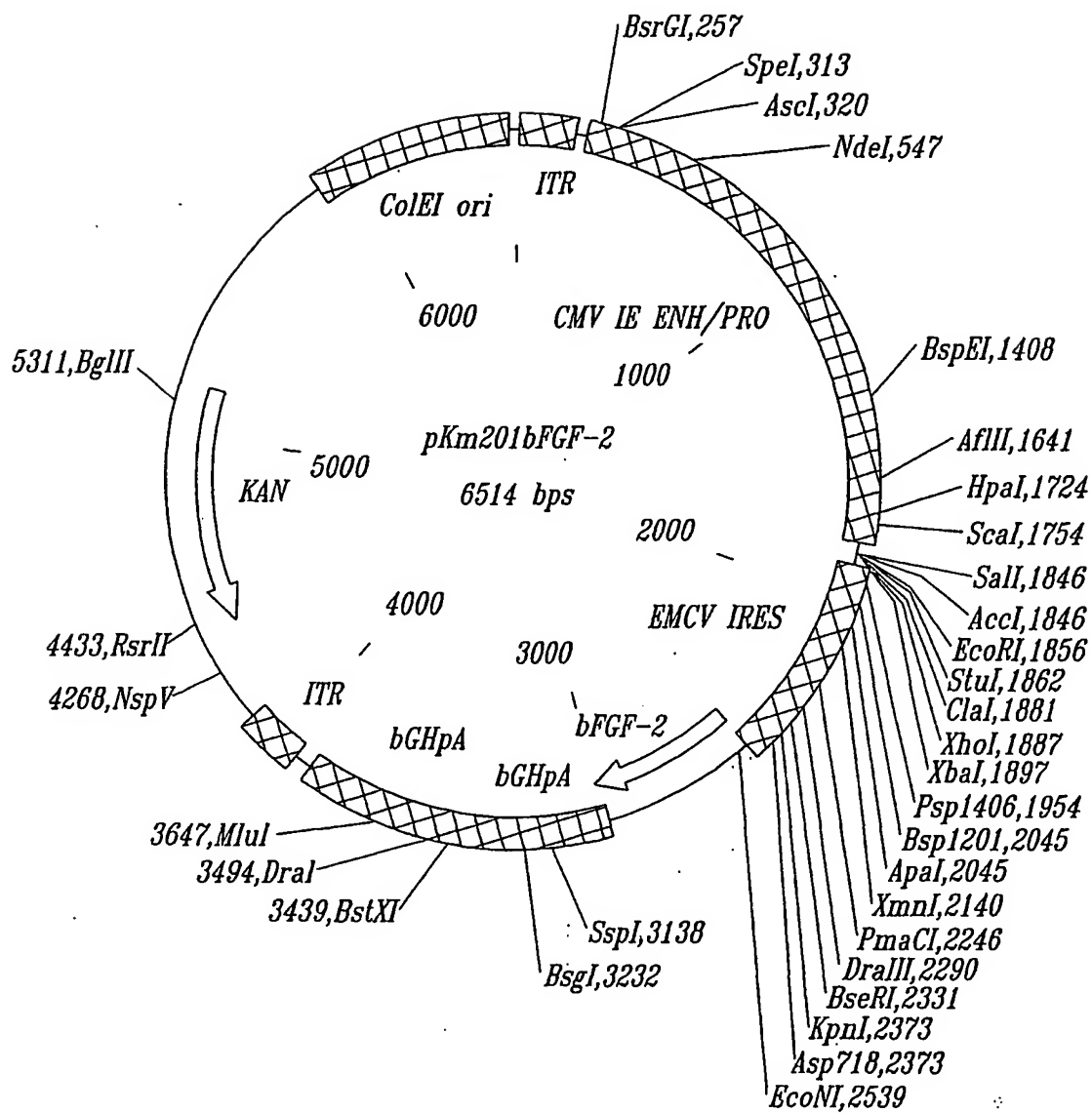


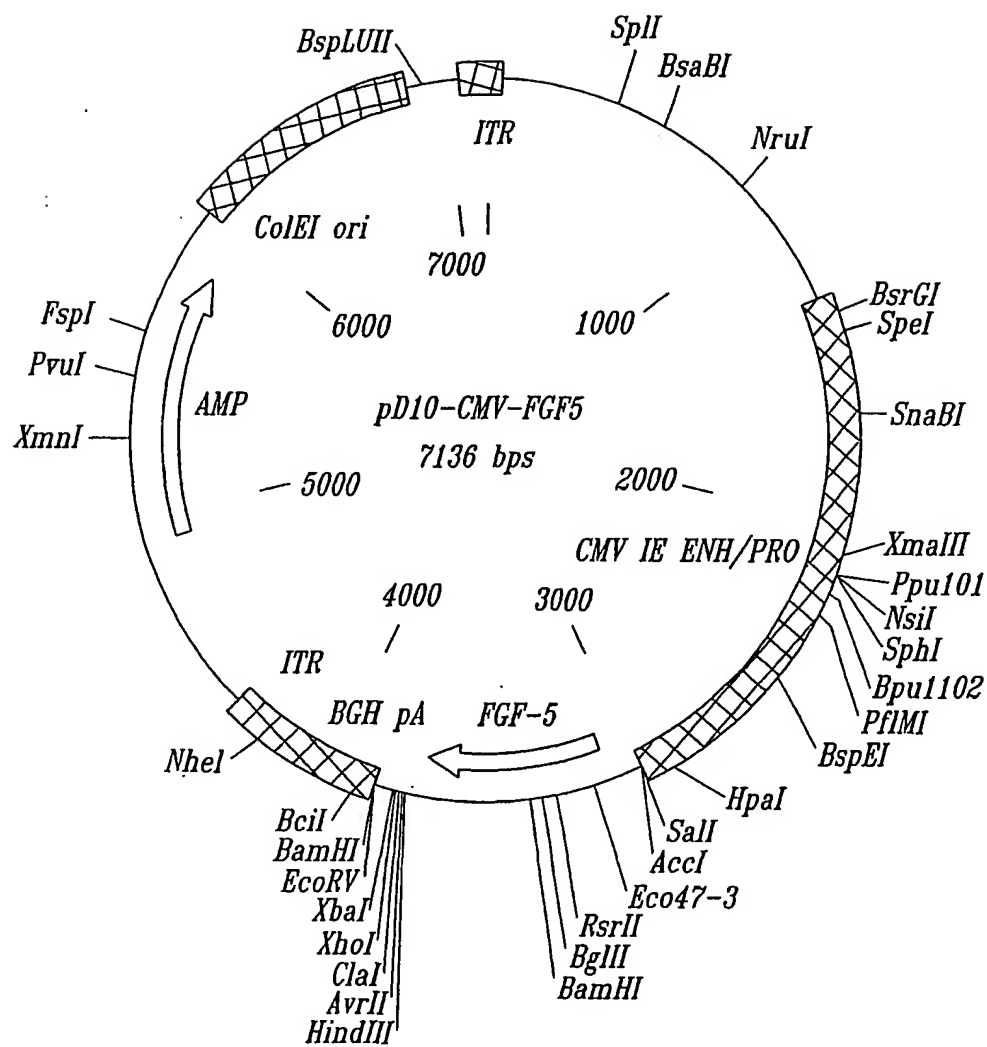
Fig. 1

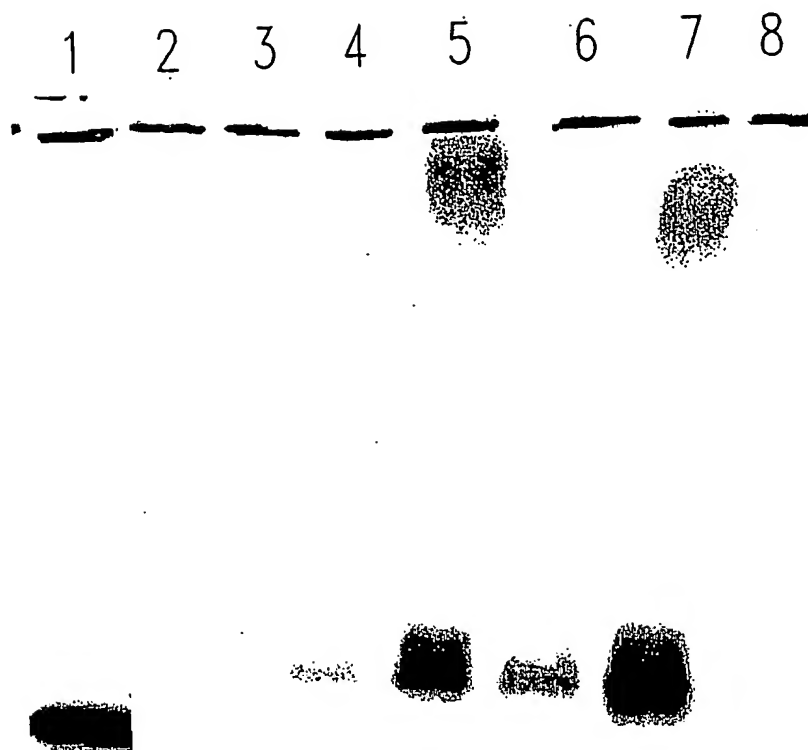
ACCATGTAGCGGCCCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGT
CGCCCGGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAAT
GATTAACCCGCCATGCTACTTATCTACGTAGCCATGCTCTAGGGAATTGGCCGCGGAATTTGACTCTAGGCCATTG
CATACGTTGTATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGACATTGATT
ATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTTACATAAC
TTACGGTAATAGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCAATTGACGTCAATAATGACGTATGTTCCCAT
GTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTACGGTAACTGCCCACTTGGCAGTACATCA
AGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACA
TGACCTTACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGG
CAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCAGTCTCCACCCCAATTGACGTCAATGGGAG
TTTGTGTTTGGCACCAAAATCAACGGGACTTTCAAAATGTCTGAATAACCCCGCCCGTTGACGCAATGGGCGGTA
GGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGC
TGTTTTGACCTCCATAGAAGACACCGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGCGGATTCC
CCGTGCCAAGAGTGACGTAAGTACCGCTATAGACTCTATAGGCACACCCCTTTGGCTCTTATGCATGCTATACTGT
TTTTGGCTTGGGGCTATACACCCCGCTCCTTATGCTATAGGTGATGGTATAGCTTAGCCTATAGGTGTGGTTAT
TGACCATTATTGACCACTCCCTATTGGTGACGATACTTTCATTACTAATCCATAACATGGCTCTTTGCCACAAC
ATCTCTATTGGCTATATGCCAATACTCTGTCTTCAGAGACTGACACGGACTCTGTATTTTACAGGATGGGTCCA
TTTATTATTTACAATTCACATATACAACACGCCGTCCCGGTGCCCGCAGTTTTTATTAACATAGCGTGGGATC
TCCGACATCTCGGGTACGTGTTCCGGACATGGGCTCTTCTCGGTAGCGCGGAGCTTCACATCCGAGCCCTGGTC
CCATCCGTCCAGCGGCTCATGGTCGCTCGGCAGCTCTTGCTCCTAACAGTGGAGGCCAGACTTAGGCACAGCACAA
TGCCCAACCACCAGTGTGCCGACAAAGCCGTGGCGGTAGGGTATGTGTCTGAAAATGAGCTCGGAGATTGGGCT
CGCACCTGGACGCAGATGGAAGACTTAAGGCAGCGGCAGAAGAAGATGCAGGCAGCTGAGTTGTTGTATTCTGATAA
GAGTCAGAGGTAACCTCCGTGCGGTGCTGTTAACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGC
GGCGCCACCAGACATAATAGCTGACAGACTAACAGACTGTCTCTTCCATGGGCTTTTCTGCAGTCACCGTCGTC
GACCTAAGAAATCAGGCCTAAGCTTCTAGGTATCGATCTCGAGCAAGTCTAGAGGGAGACCACAACGGTTCCCTC
TAGCGGGATCAATTCGCCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCT
ATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTGTCTCTTGACGAGC
ATTCCTAGGGGCTTTTCCCTCTCGCCAAGGAATGCAAGGCTGTTGAATGTGCTGAAGGAAGCAGTTCCTCTGGA
AGCTTCTTGAAGACAAACAGTGTGTAGCGACCCTTTGACGGCAGCGGAACCCCACTGGCGACAGGTGCTCT
GGGGCCAAAGCCACGTGTATAAGATACACCTGCAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTT
GTGGAAGAGTCAAATGGCTCTCTCAAGCGTATTCAACAAGGGCTGAAGGATGCCAGAAGGTACCCATTGTAT
GGGATCTGATCTGGGCTCGGTGCACATGCTTACATGTGTTAGTCGAGGTAAAAAACGTCTAGGCCCCCGGA
ACCAGGGGACGTGTTTTCTTTGAAAAACAGATAATACCATGGCCGCCGGGAGCATCACACGCTGCCAGCCCT
GCCGGAGGACGGCGGCAGGGCGCTTTCCGCCGGGCCACTTCAAGGACCCCAAGCGCTGTACTGCAAGAACGGG
GCTTCTTCTGCGCATCCACCCGACGGCCGAGTGGACGGGTCGCGAGAAGAGCGACCCACACATCAAACACAA
CTTCAAGCAGAAGAGAGAGGGTTGTGTCTATCAAGGAGTGTGTGCAACCGTTACCTTGCTATGAAAGAAGATGG
AAGATTACTAGCTTCTAAATGTGTTACAGACGAGTGTCTTTTGAACGATTGGAGTCTAATACTACAATACTT
ACCGGTCAAGGAAATACACAGTTGGTATGTGGCACTGAAACGAACCTGGGAGTATAAATTTGGATCCAAACAGGA
CCTGGGAGAAAGCTATACTTTTCTTCCAATGTCTGCTAAGAGCTGATCTAATGGCAGCATCTGATCTCATTTTA
CATGAAGCTGGTGGCATCCCTGTGACCCCTCCCACTGCTCTCTGCGCTGGAAGTTGCCACTCCAGTGCCACC
AGCCTTGCTCTAATAAAATTAAGTTGCATCATTTGTCTGACTAGGTGTCTTCTATAATATTATGGGGTGGAGGG
GGTGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCTCGCGGGTCTATTGGGAACCAAGCTGGAGTG
CAGTGGCACAACTTGGCTCACTGCAATCTCCGCTCCTGGGTTCAAGCGATTCTCTGCTCAGCCTCCCGAGTGT

Fig. 2A

TTGGGATTCAGGCATGCATGACCAGGCTCAGCTAATTTTGTGTTTTTGGTAGAGACGGGGTTTACCATATTGGC
CAGGCTGGTCTCCAACCTCCTAATCTCAGGTGATCTACCCACCTTGGCCTCCCAAATTGCTGGGATTACAGGCGTGAA
CCTGCTGCTCCCTTCCCTGCTCTTCTGATTTTAAATAACTATACCAGCAGGAGGACGTCCAGACACAGCATAGGCTA
CCTGGCCATGCCCAACCGGTGGGACATTTGAGTTGCTTGCTTGSCACTGTCTCTCATGCGTTGGGTCCACTCAGTA
GATGCCGTGTGAATTATCGGATCCACTACGCGTTAGAGCTCGCTGATCAGCCTCGACTGTGCCCTTCTAGTTGCCAGC
CATCTGTTGTTTGGCCCTCCCGGTGCCCTTCTTGACCCTGGAAGGTGCCACTCCACTGTCTTTCTAATAAAAT
GAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGA
GGATTGGGAAGACAATAGCAGGGGGTGGGCGAAGAACTCCAGCATGAGATCCCGCGCTGGAGGATCATCCAGCCA
ATTCCTTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAG
TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCGGGCGACCAAAGGTGCGCCGACGCCCGGGCTTTGC
CCGGGCGGCTCAGTGAGCGAGCGAGCGCGAGGGGTGGGCGAAGAACTCCAGCATGAGATCCCGCGCTGGAGGA
TCATCCAGCCGCGCTCCCGAAAAACGATTCCGAAGCCCAACCTTTCATAGAAGGCGGCGGTGGAATCGAAATCTCGT
GATGGCAGGTGGGGCTCGCTTGGTGGTTCATTTCGAACCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGA
TAGAAGGCGATGCGCTGCGAATCGGGAGCGCGATACCGTAAAGCACGAGGAAGCGGTGAGCCATTGCGCGCAAG
CTCTTCAGCAATATCAGGGTAGCCAACGCTATGTCTGATAGCGGTCCGCCACCCAGCCGGCCACAGTCGATGA
ATCCAGAAAAGCGGCATTTTCCACCATGATATTGGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTCGCCG
TCGGGCGATGCGCGCTTGAGCTGGCGAACAGTTCGGCTGGCGGAGCCCTGATGCTCTTCGTCCAGATCATCTG
ATCGACAAGACCGGCTTCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTGAATGGGCGAGTAG
CCGGATCAAGCGTATGCAGCCGCGCATTTGCATCAGCCATGATGGATACTTCTCGGCAGGAGCAAGGTGAGATGAC
AGGAGATCCTGCGCCGCGACTTCGCCCAATAGCAGCCAGTCCCTTCCCGCTCAGTGACAACGTCGAGCACAGTCG
GCAAGGAACGCCGCTCGTGGCCAGCCACGATAGCGCGCTGCCCTCGTCTGCAGTTTCATTAGGGCACCGGACAGGT
CGGCTTCGACAAAAAGAACCGGGCGCCCTGCGCTGACAGCCGGAACAGGCGGCATCAGAGCAGCCGATTGTCTGT
TGTGCCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCGGAGAACCTGCGTGCAATCCATCTTGTTCATCAT
GCGAAACGATCCTCATCTGTCTCTTGATCAGATCTTGATCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCA
TCCAGTTTACTTTGACGGGCTTCCAACCTTACCAGAGGGCGCCAGCTGGCAATTCCGGTTTCGCTTGTGTCAT
AAAACCGCCAGTCTAGCTATCGCCATGTAAAGCCACTGCAAGCTACCTGCTTTCTCTTTCGCGTTGCGTTTCCCT
TGTCCAGATAGCCAGTAGCTGACATTCATCCGGGTGAGCACCGTTTCTGCGGACTGGCTTTCTACGTGTTCCGT
TCCTTTAGCAGCCCTTGGCCCTGAGTGTGCGGCGAGCGTGAAGCTGTCAATTCCGCGTAAATTTTGTAAATC
AGCTCATTTTAAACCAATAGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGCCCGAGATAGGGTTGAG
TGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCGGATCAGCTTATGCGGTGTAAATACCGCACAGATGCGTAAGGAGAAAAACCGCATCAGGCGCTC
TTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCCGCTGCGGCGAGCGGTATCAGTCACTCAAAGGCGG
TAATACGGTTATCCACAGAATCAGGGGATAACGAGGAAGAACATGTAGCAAAAGGCCAGCAAAAGGCCAGGAAC
CGTAAAAAGGCGCGTGTGCTGGCGTTTTCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAGATACAGGCGTTTCCCTTGAAGCTCCCTCGTGCGCTCTCTG
TTCCGACCTTCCGCTTACCGGATACCTGTCCGCTTCTCCCTTCCGGAAGCGTGGCGCTTCTCATAGCTCACGC
TGATAGTATCTCAGTTCCGTTAGGTGTTGCTCCAAGCTGGGCTGTGTGCAGAACCCCCGTTTCAGCCGACCG
CTGGCCCTTATCCGGTAACATCGTCTTGAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG
GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACCT
AGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGTTGGTAGCTCTTGATCCGG
CAAAACAACACCGCTGGTAGCGCGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGA
AGATCCTTTGATCTTTCTTACTGAACGGTGATCCCAACCGGAATT

Fig. 2B

*Fig. 3*

*Fig. 4*

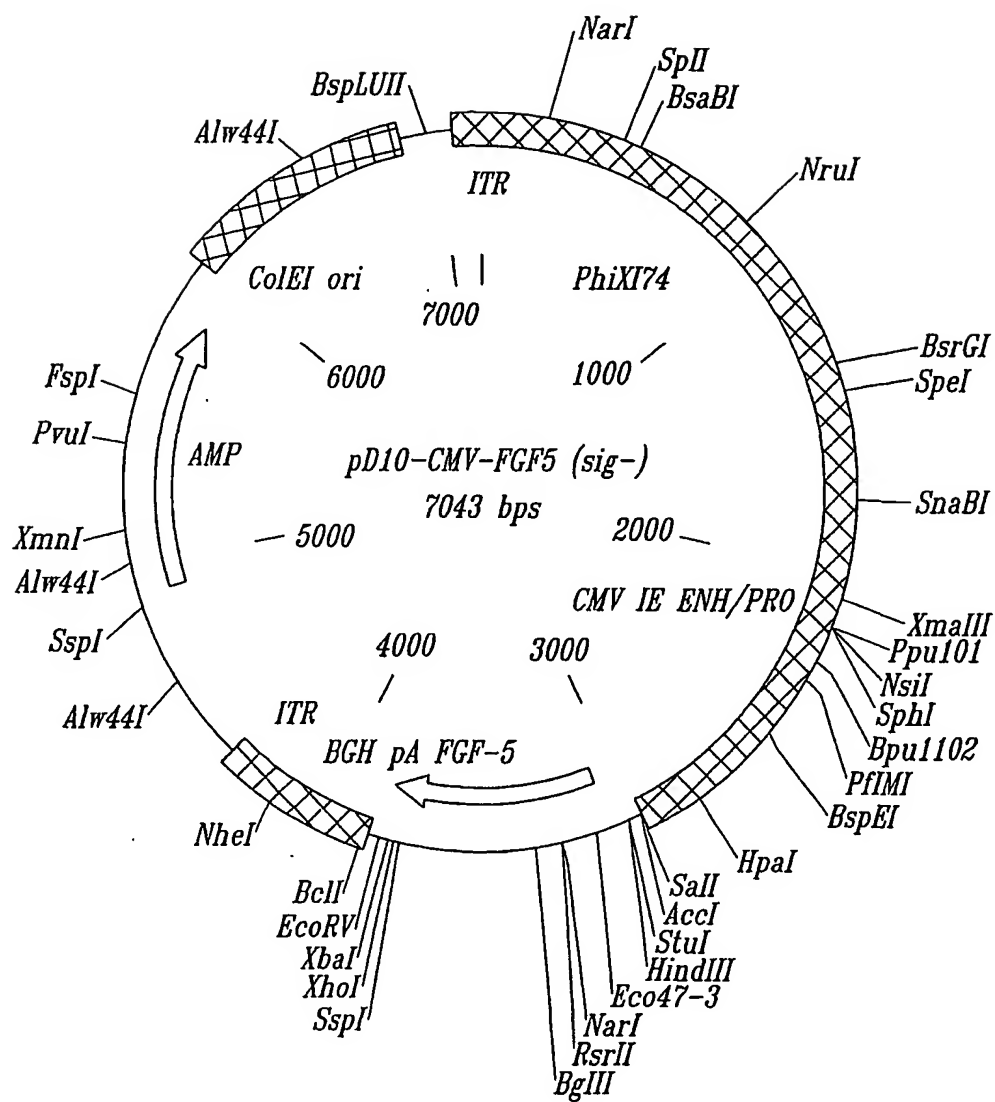
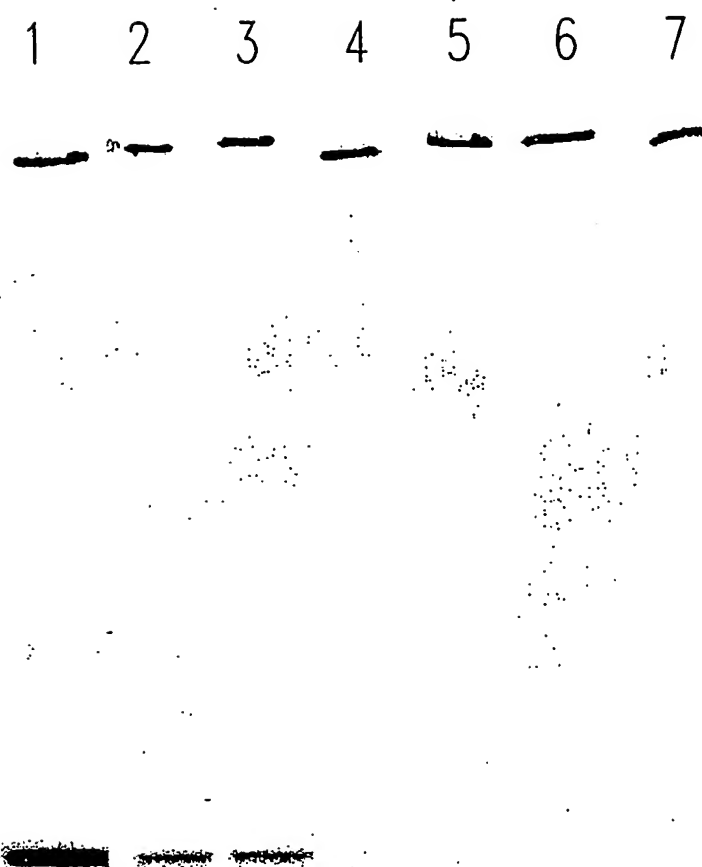


Fig. 5

*Fig. 6*

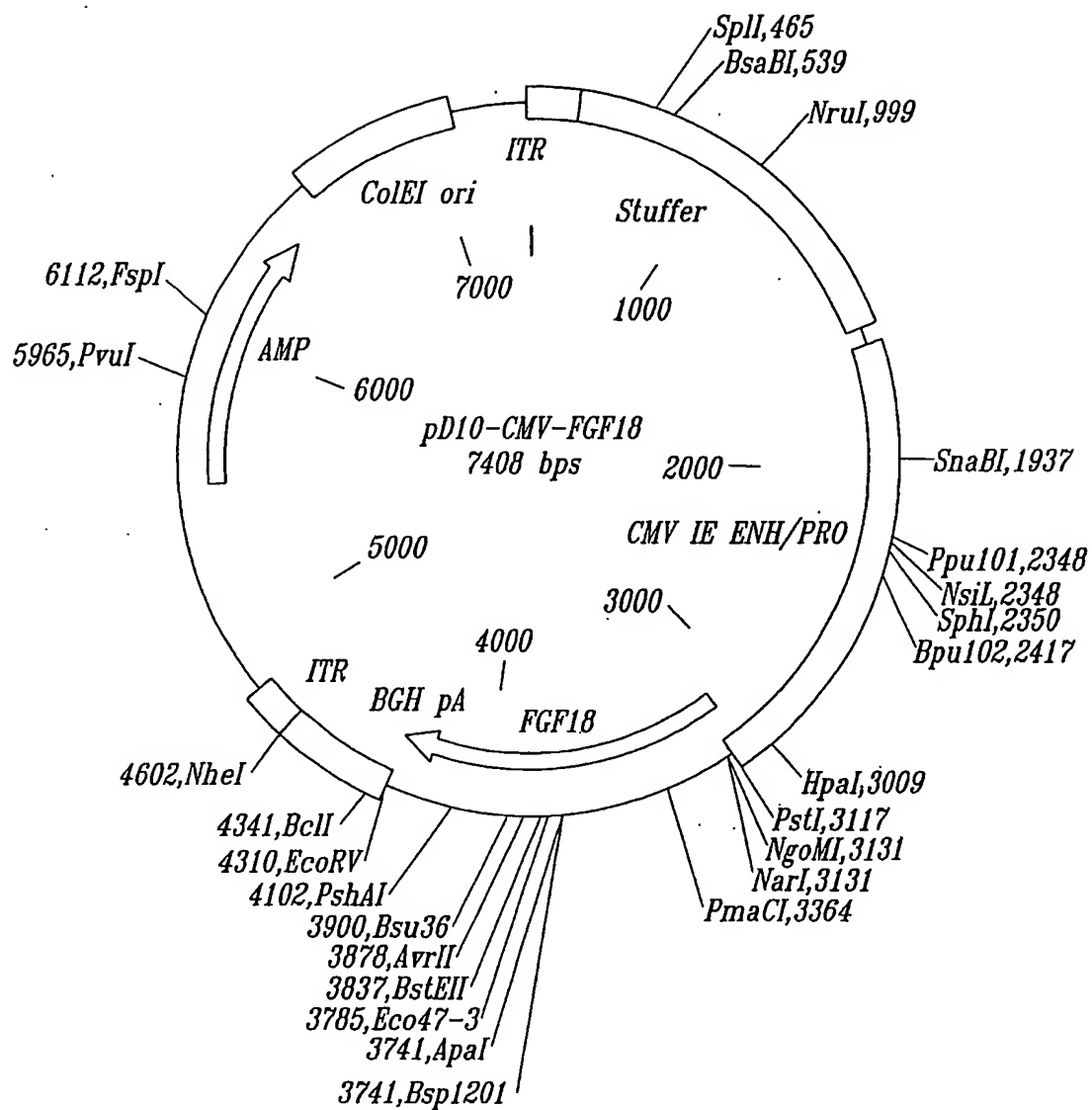
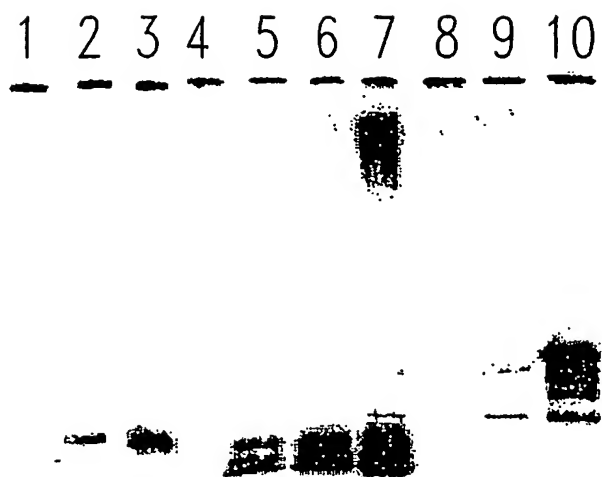


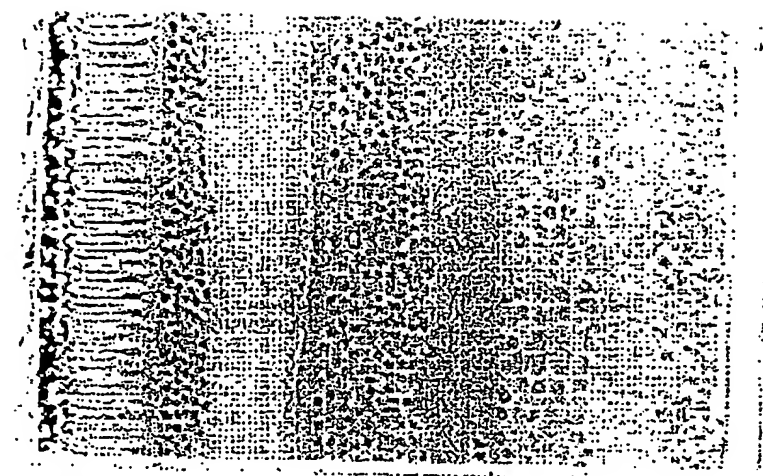
Fig. 7

*Fig. 8*



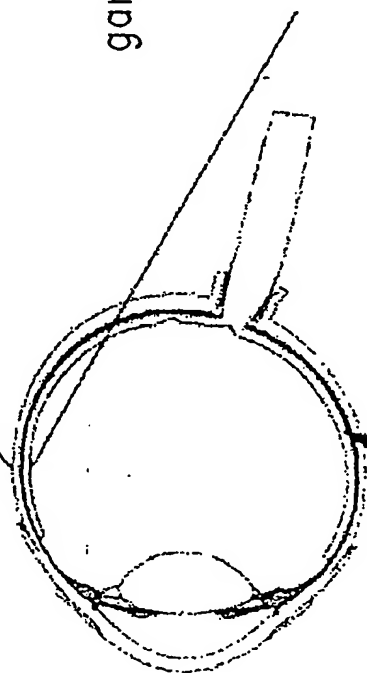
BEST AVAILABLE COPY

Fig. 9



OUTER
SEGMENTS —
INNER
OUTER NUCLEAR LAYER
(photoreceptors)

ganglion cells



RETINA

Fig. 10

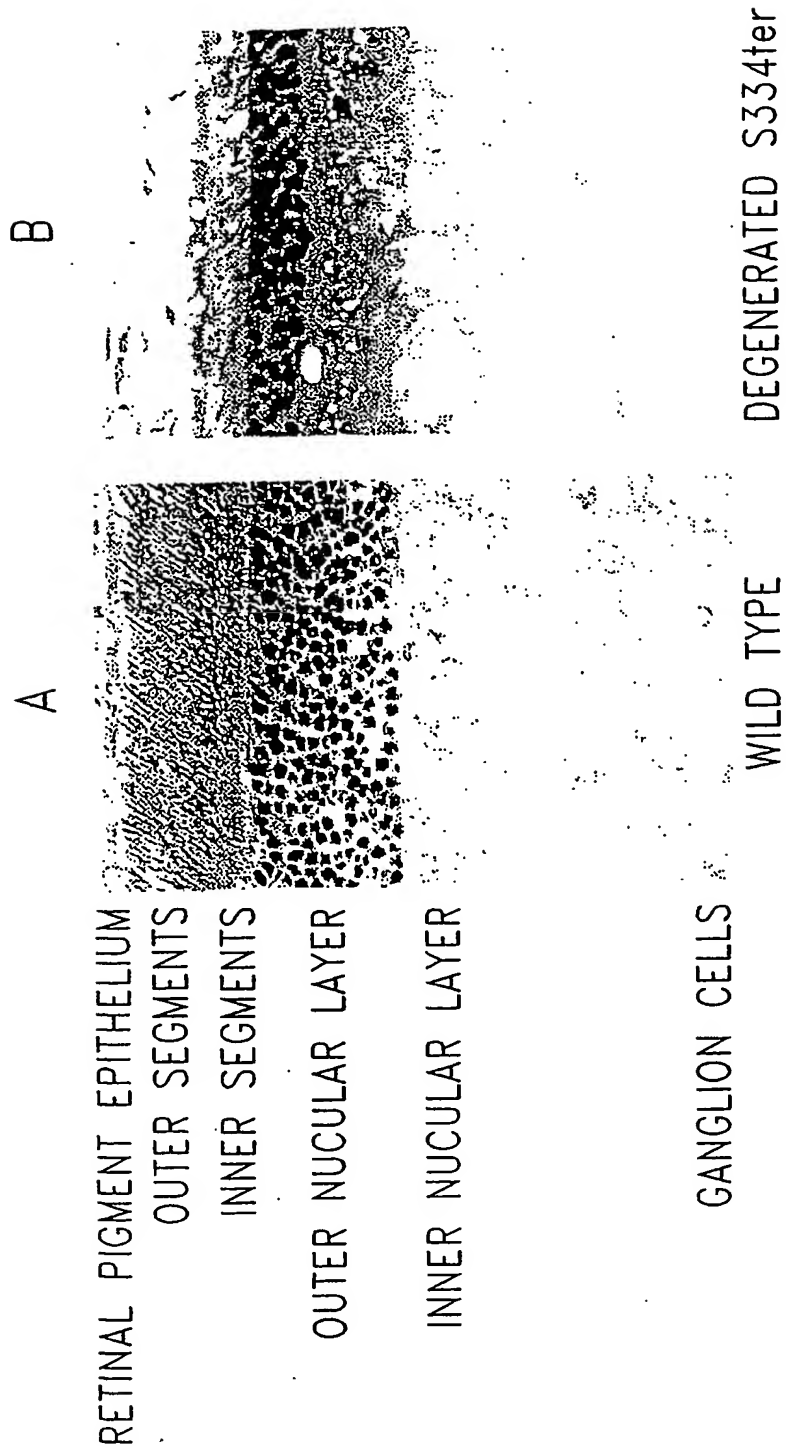


Fig. 11

BEST AVAILABLE COPY

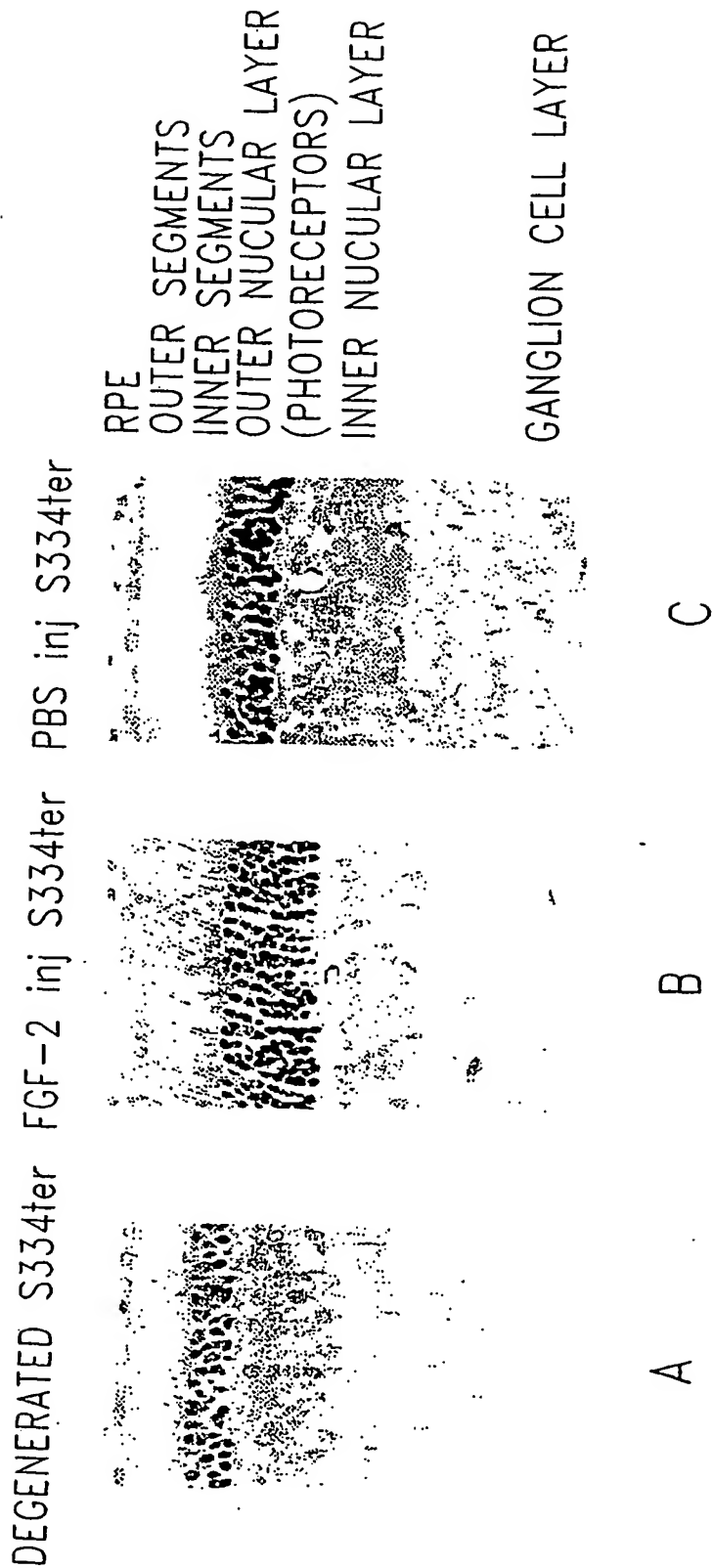


Fig. 12

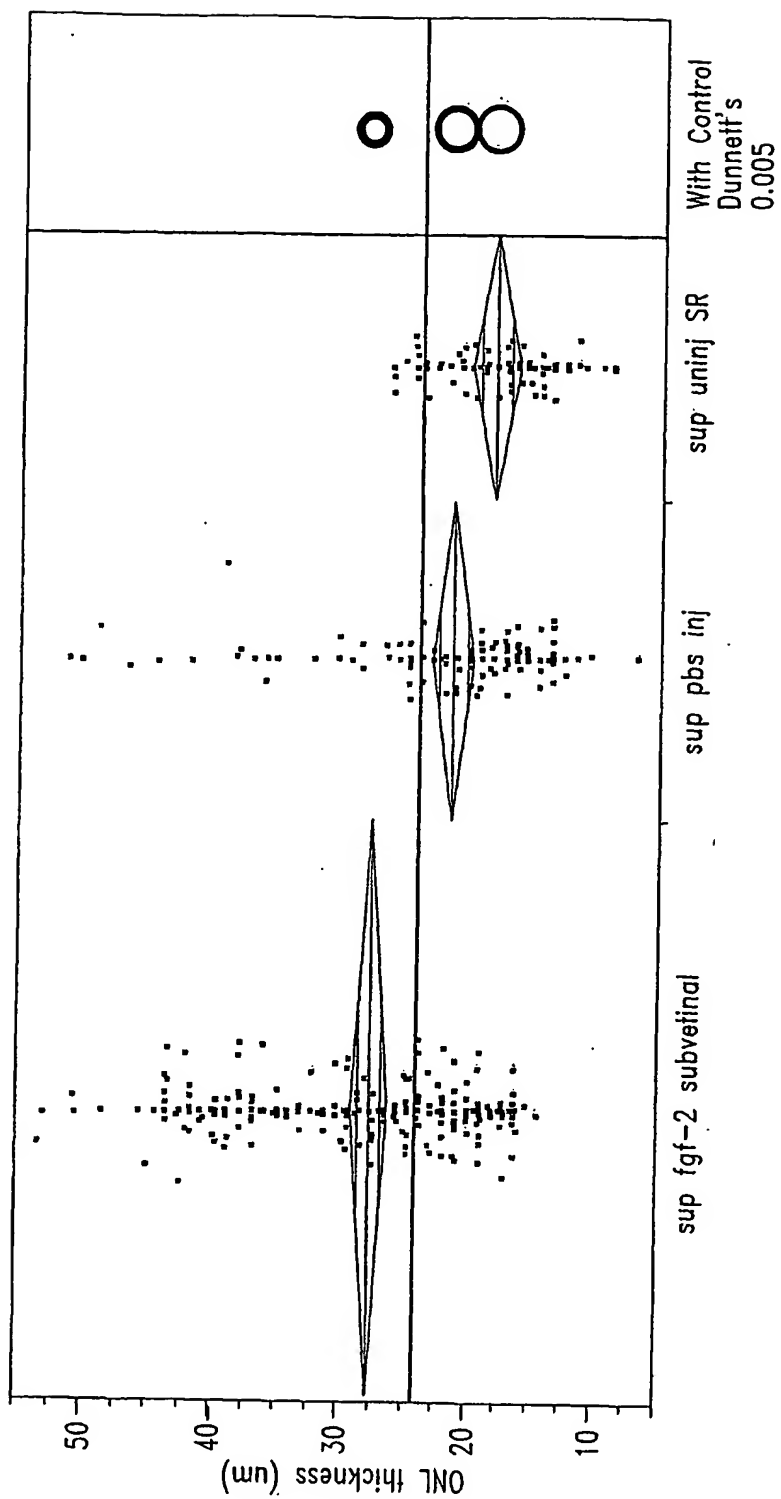
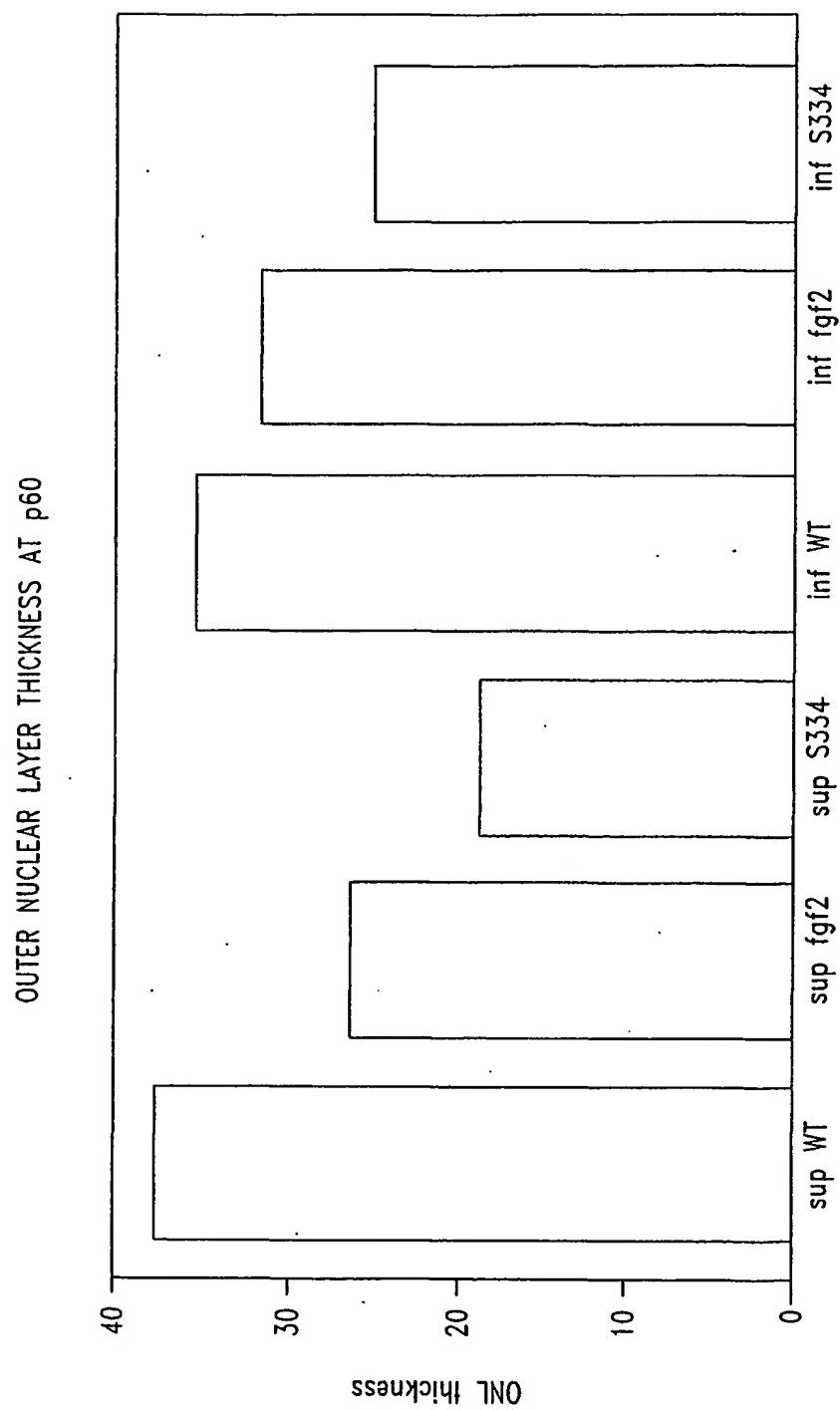


Fig. 13

*Fig. 14*

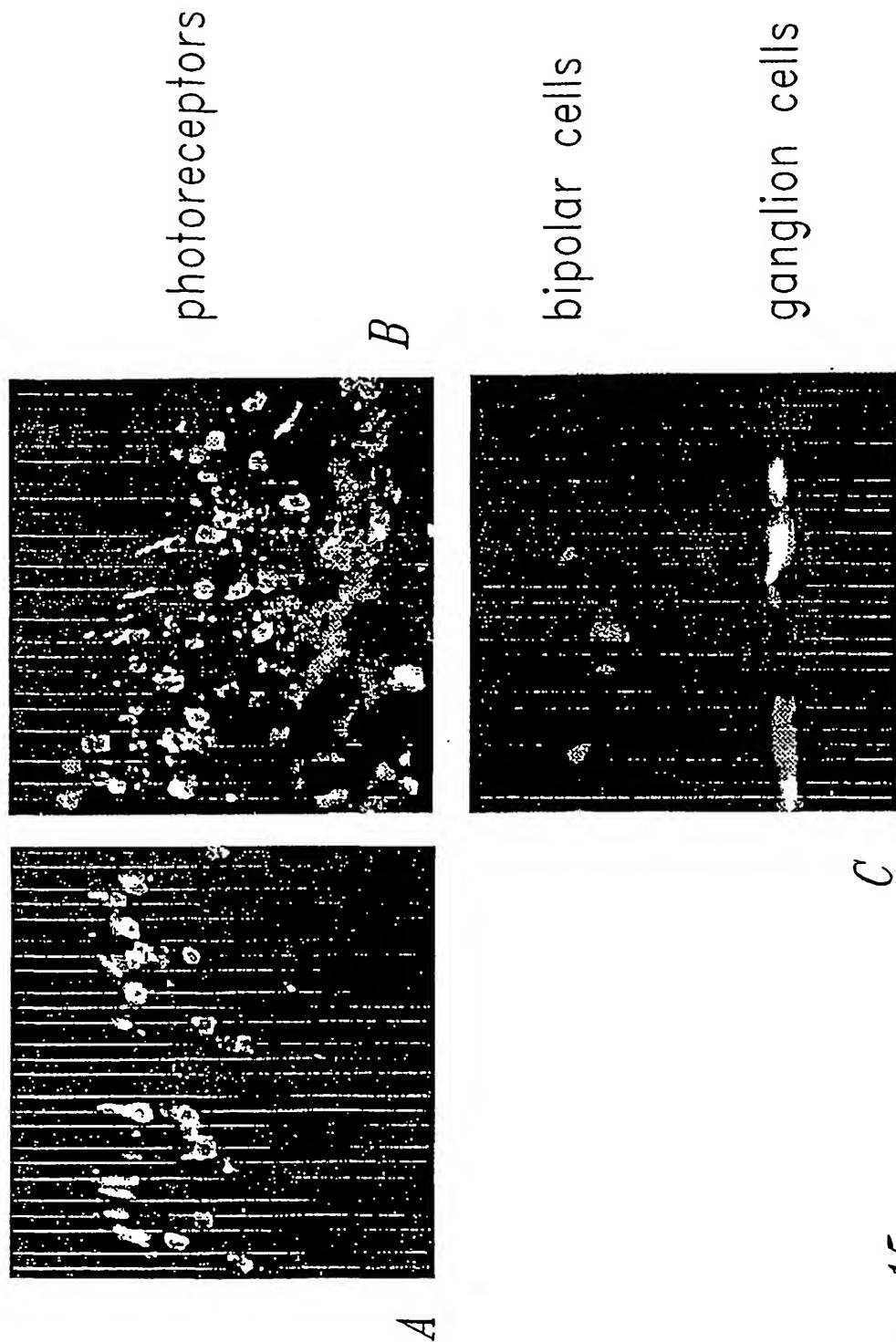
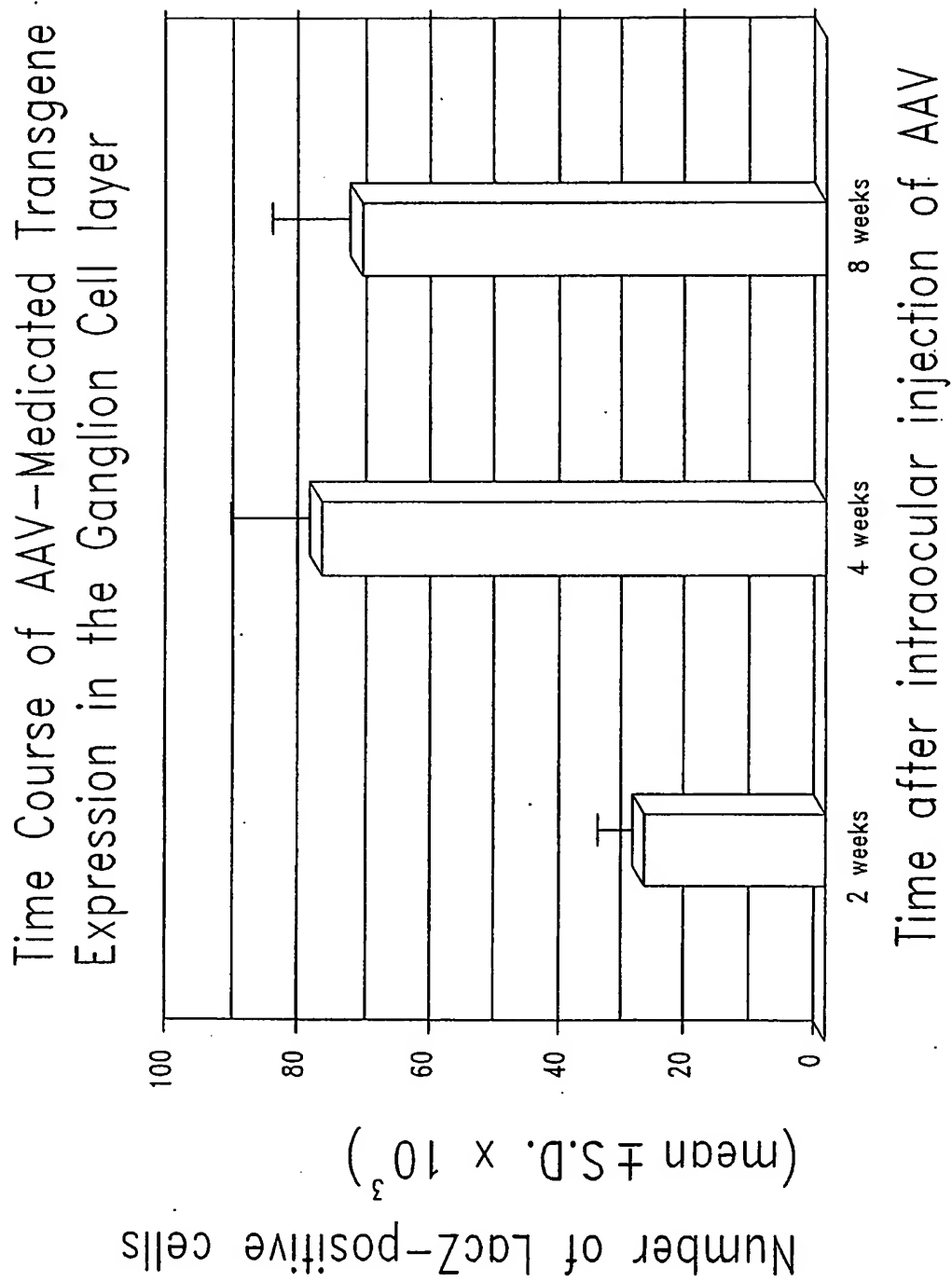


Fig. 15

AAV-LacZ Transduction of Retinal Ganglia



Fig. 16

*Fig. 17*

Localization of AAV-Medicated LacZ Gene Product
in Retrograde Labeled RCG

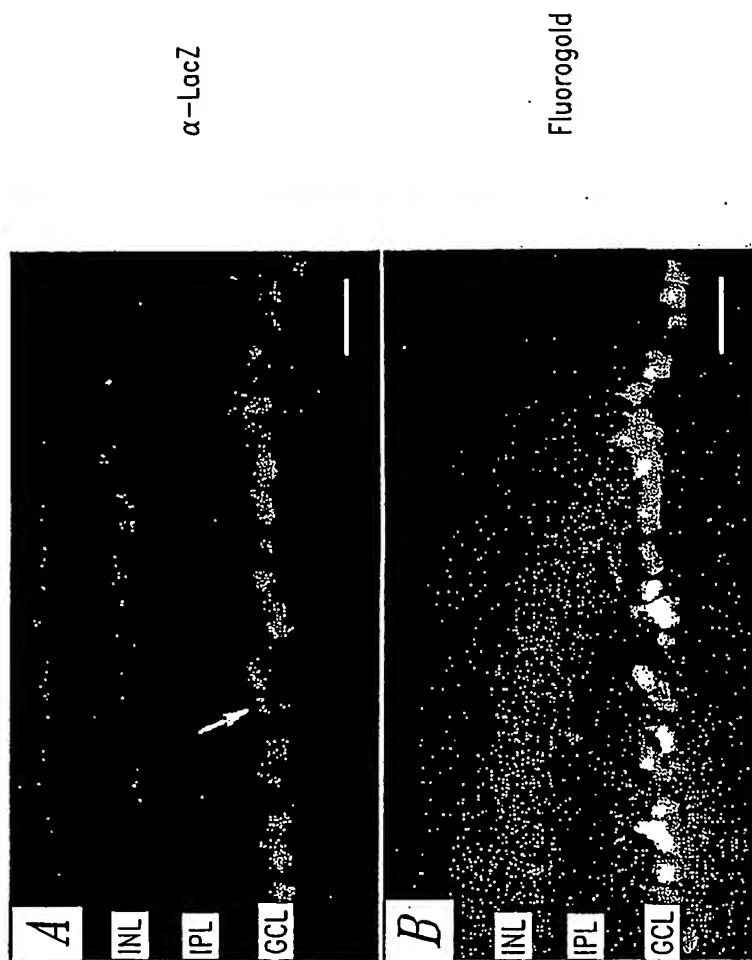


Fig. 18

Quantification of Flourogold and LacZ Positive
Cells in the Ganglion Cell Layer Following
Intravitreal Injection of rAAV-LacZ

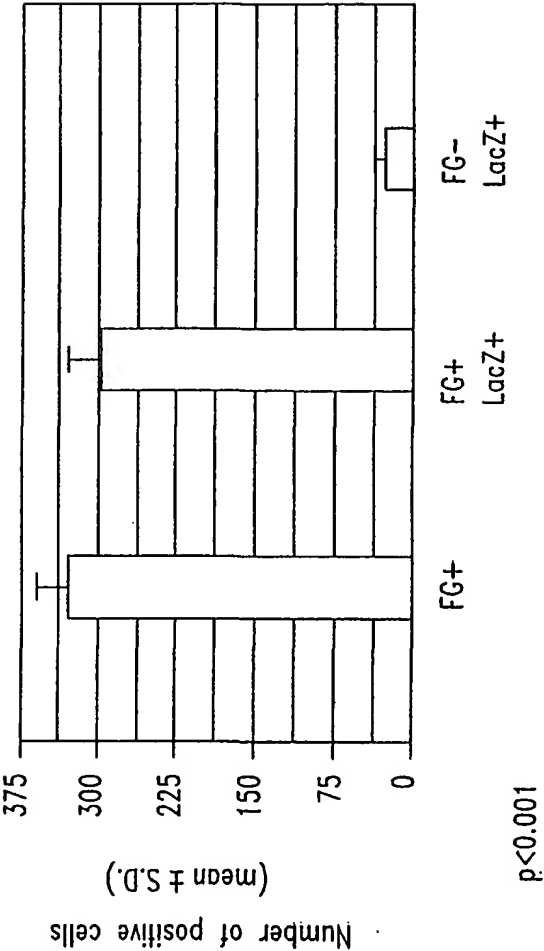


Fig. 19

Localization of Heparin sulfate Proteoglycan, the Cellular
Receptor for AAV, in the Adult Rat Retina

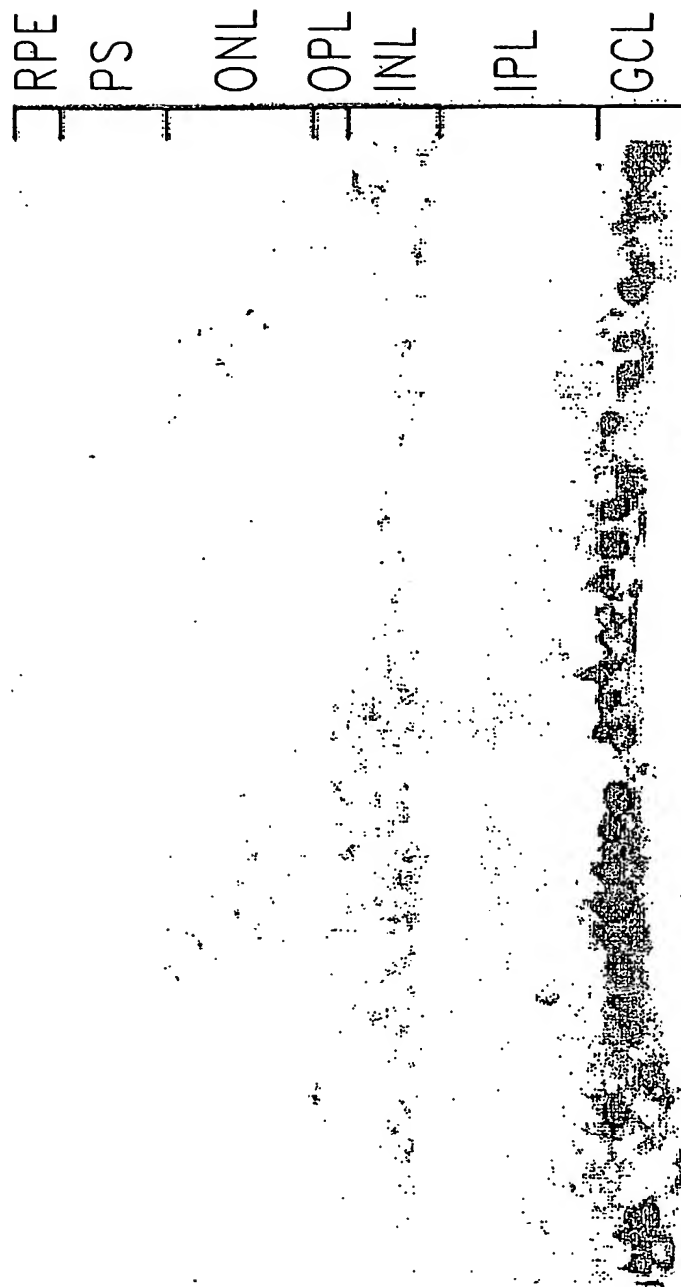


Fig. 20

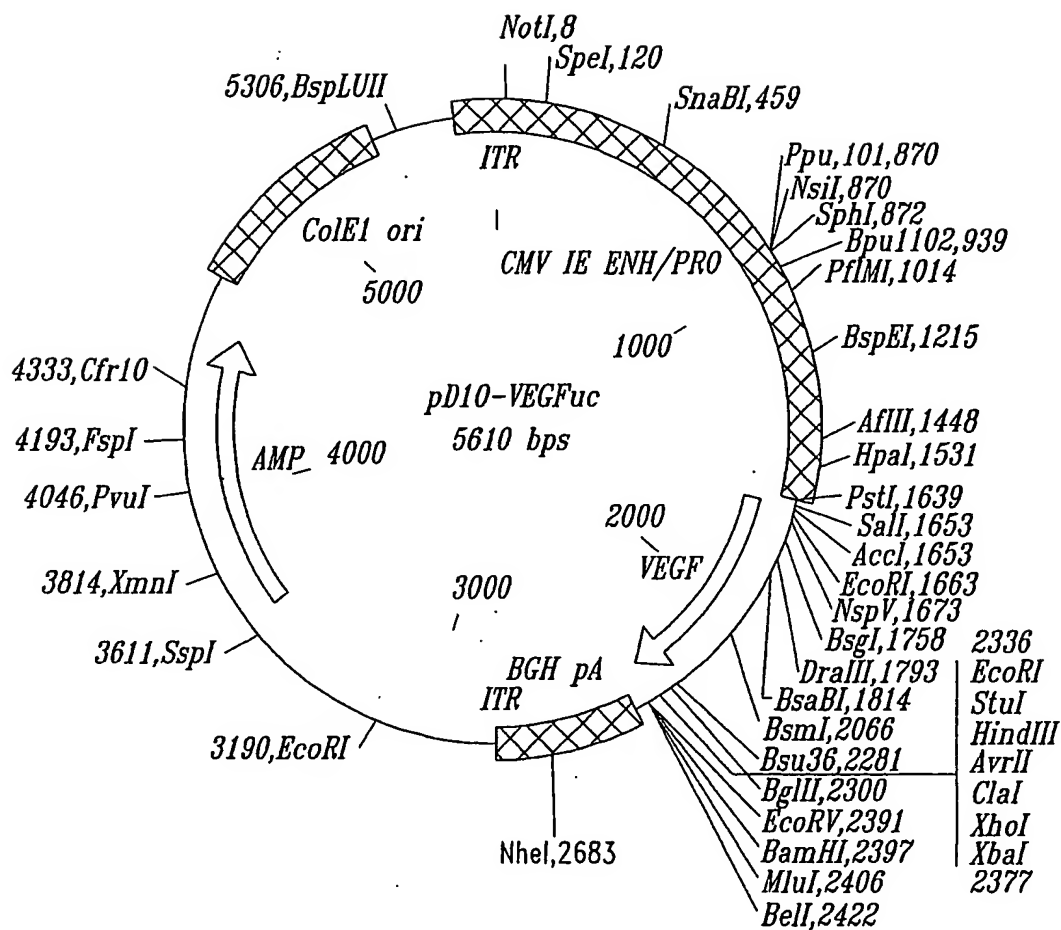


Fig. 21

Nucleotide Sequence of pD10-VEGFuc

AAAACTTGGCGCCGCGGAATTCGACTCTAGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACGCCA
TGTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAA
TGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAAT
GGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCTATTGACGTCAATGACGTAATGGCCGCGC
TGGCATTATGCCAGTACATGACCTTACGGGACTTCTACTTGGCAGTACATCTAGCTATTAGTCATCGTATTACCATGGTGATGCGGTTTTGGCAGTA
CACCATGGGCGTGGATAGCGGTTTGACTCAGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACT
TTCCAAAATGTCGAATAACCCGCGCCGTTGACGCAATGGGCGTAGGGGTGACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAG
ATCGCTGGAGACGCCATCCAGCTGTTTTGACCTCCATAGAAGACACGGGACCGATCCAGCTCCGCGGCCGGGAACGGTGCAATGGAACGCGGATTCC
CCGTGCCAAGAGTGACGTAAGTACCGCTATAGACTCTATAGGCACACCCCTTGGCTCTTATGCATGCTATACGTGTTTTGGCTTGGGCGCTATACACC
CCGCTCCTTATGCTATAGGTGATGGTATAGCTTAGCCTATAGGTGTTGGTTATTGACCATTATTGACCACTCCCTATTGGTGACGATACTTCCATTACT
AATCCATAACATGGCTCTTTGCCACAATATCTCTATTGGCTATATGCCAATACTCTGTCCTCAGAGACTGACACGGACTCTGTATTTTACAGGATGG
GTCCATTTATTTACAAATTCACATATACAACAACGCGTCCCGGTGCCGAGTTTTTATTAACATAGCGTGGGATCTCCGACATCTCGGTACGT
GTTCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAGCTTCCACATCCGAGCCCTGGTCCATCCGTCAGCGGCTCATGGTCGCTCGGACGCTCCTTGCTC
CTAACAGTGGAGGCCAGACTTAGGCACAGCAATGCCACCACCACAGTGTGCCGACAAGGCCGTGGCGTAGGGTATGTGTCTGAAAATGAGCTCGG
AGATTGGGCTCGCACCCTGGACGCGAGATGGAAGACTTAAGGCAGCGGCAGAAGAGATGCAGGCGAGTGAAGTTGTGATTCTGATAAGAGTCAGAGGTAAC
TCCGTTGGGTGCTGTTAACGGTGGAGGSCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGGCCACCAGACATAATAGCTGACAGACTAACAGAC
TGTTCCCTTTCATGGGTCTTTCTGCACTCACCGTCGTCGACCTAAGAAATCGCCCTTCGAAACCATGAATTTCTGCTGTCTTGGGTGCATTGGAGCCCT
GCCCTGTGCTCTACCTCCACCATGCCAAGTGGTCCAGGCTGCACCATGGCAGAAGGAGGAGGCGAGAATCATCAGAAAGTGGTGAAGTTCATGATGT
CTATCAGCGCAGTACTGCCATCCAATCGAGACCTGGTGGACATCTCCAGGAGTACCTGATGAGATCGAGTACATCTTAAGCCATCCTGTGTGCC
TGATGCGATGCGGGGCTGCTGCAATGACGAGGGCTGGAGTGTGTGCCACTGAGGAGTCCAACATCACCATGCGATTATGCGGATCAAACCTCACC
GGCCAGCACATAGGAGAGATGAGCTTCTACAGCACAACAATGTGAATGCAGACCAAGAAAGATAGAGCAAGACAAGAAAATCCCTGTGGGCTTGCTC
AGAGCGGAGAAAGCATTTGTTGTACAAGATCCGAGACGTGAATGTTCTGCAAAAACACAGACTCGCGTTGCAAGGCGAGGCGAGCTTGAAGTAAACG
AACGTACTTGAGATGTGACAAGCCGAGGCGGTGAGCCGGGCGAGGAGGAGGAGGCTCCCTCAGGGTTTCGGGAACCATGATCTCTACCGAGAAAGACTGA
TACAGAAAGGCGAATTCAGGCTAAGCTTCTAGGTATCGATCTGAGCAAGTCTAGAAAGCCATGGATATCGGATCCACTACGCGTTAGAGCTCGTG
TCAGCTCGACTGTGCTTCTAGTTGCCAGCATCTGTTGTTGCCCTCCCGGTGCTTCTTGACCTGGAAGGTGCCACTCCCACTGTCTTTCTTA
ATAAATGAGGAAATGCATCGATTGTCTGAGTAGGTGCTATTCTATTGCGGGGTGGGTGGGCGAGGACAGCAAGGGGAGGATTGGGAAGACAATA
GCAGGGGGGTGGGCGAAGAACTCCAGCATGAGATCCCGCGCTGGAGGATCATCCAGCTAGCAAGTCCATCAGTGATGGAGTTGGCACTCCCTCTCTGC
GCGCTCGCTCGCTCACTGAGGCGGGGCGACCAAGGTGCGCCGACGCGCGGCTTTGCGCGGCGGCTCAGTGAGCGAGCGAGCGCCAGCGATTCTCT
TGTTTGTCCAGACTCTCAGGCAATGACCTGATAGCTTTGTAGAGACCTCTCAAAAATAGCTACCTCTCCGGCATGAATTTATCAGCTAGAACGGTTGA
ATATCATATTGATGGTGAATTTGACTGTCTCCGGCTTTCTACCCGTTGAATCTTTACCTACACATTACTCAGGCATTGCAATTTAAATATATAGGGTT
CTAAAAATTTTATCCTTGCGTTGAAATAAAGGCTTCTCCGCAAAAGTATTACAGGGTCATAATGTTTTTGGTACAACCGATTAGCTTTATGCTCTGAG
GCTTTATGCTTAATTTTGTAAATCTTTGCTTGCTGTATGATTTATTGGATGTTGGAATTCCTGATGCGGTAATTTCTCTTACGCATCTGTGCGGTA
TTTACACCGCATATGGTGACTCTCAGTACAATCTGCTCTGATGCCGATAGTTAAGCCAGCCCGACCCGCAACACCCGCTGACGCGCCTGACGG
GCTTGTCTGCTCCCGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGTTTACCGTCATCACCAGAACGCGCGAGACG
AAAGGGCTCGTGATACGCTATTTTATAGGTTAATGTATGATAAATGGTTTCTTAGACGTGAGTGGCACTTTTCGGGAAATGTGCGCGGAACCC
CTATTTGTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATTTGAAAAAGGAGAGTATGAGT

Fig. 22A

ATTCAACATTTCCGTGTCGCCCTTATCCCTTTTTGCGGCATTTTGCTTCCTGTTTTGCTCACCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGA
 TCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTGCGCCCGAAGAAGCTTTTCCAATGATGAGCACTT
 TTAAGTTCTGCTATGTGGCGCGGTATTATCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTAC
 TCACCACTCACAGAAAAGCATTTACGGATGGCATGACAGTAAGAGAATTATGCACTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCT
 GACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTGCACAACATGGGGGATCATGTAACTCGCCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCA
 TACCAACGACGAGCGTGACACCAGATGCCGTGAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAA
 TTAATAGACTGGATGGAGCGGATAAAGTTGCAGGACCATTCTGCGCTCGGCCCTCCGGCTGGCTGGTTATTGCTGATAAATCTGGAGCCGGTGAGCG
 TGGGTCTCGCGGTATCATTCAGCACTGGGGCCAGATGGAAGCCCTCCGCTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAA
 ATAGACAGATCGCTGAGATAGGTGCTCACTGATTAAGCATTGGTAACGTGACACCAAGTTACTCATATATACTTTAGATTGATTTAAACCTTCATTTT
 TAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTGTTCCACTGAGCGTCAGACCCGTAGAAAA
 GATCAAAGGATCTTCTTGAGATCCTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACCCGCTACCAGCGGTGGTTTGTGGCGGATCAAG
 AGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAACTACTGCTCTCTAGTGTAGCCGTAGTTAGGCCACCCTTCAAGAAC
 TCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATA
 GTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTCACACAGCCAGCTTGAGCGAAGCAGCTACACCGAAGTACCTACAGCGTG
 AGCTATGAGAAAGCGCCAGCTTCCGAAGGAGAAAGGCGGACAGGTATCCGTAAGCGGCGAGGTGGAACAGGAGAGCGCAGAGGGAGCTTCCAGGG
 GGAACGCTGGTATCTTTATAGTCTGTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGCGGAGCTATGAAAAA
 CGCCAGCAACGCGCCTTTTACGGTTCCTGGCTTTTGTGGCTTTTGCTCACATGTTCTTCTGCGTTATCCCTGATTCGTGGATAACCGTATTA
 CCGCCTTTGAGTGAGCTGATACCGCTCGCCGCGAGCGAAGCAGCGAGTCAAGTGAAGCGAAGCGAAGAGCGCCCAATACGCAACCGCCT
 CTCGCCGCGGTTGGCGGATTATTAATGACAGCTGGCGGCTCGCTCGCTCACTGAGGCCGCCCGGCAAGCCGGGCGTGGGGGACCTTTGGTCGCC
 GGCCTCAGTGAGCGAGCGCGCGCAGAGAGGAGTGCCAACTCCATCACTGAT

Fig. 22B

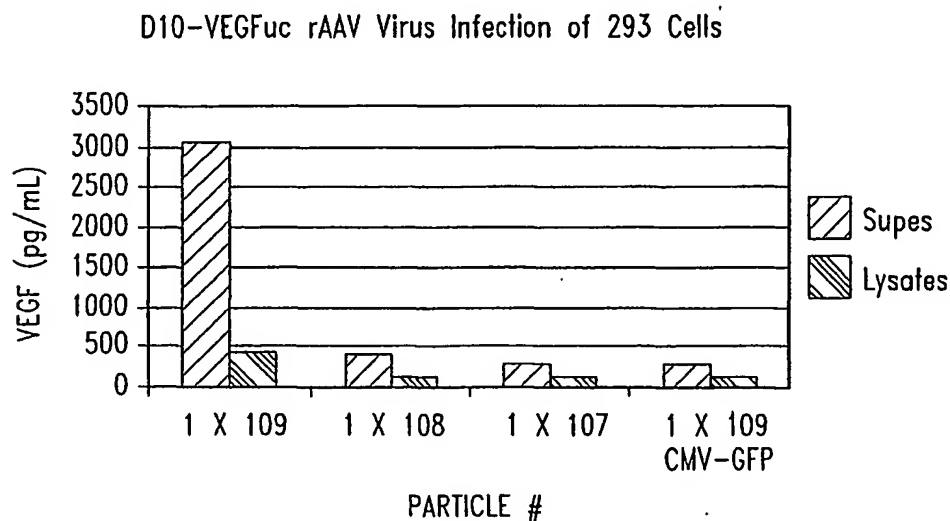
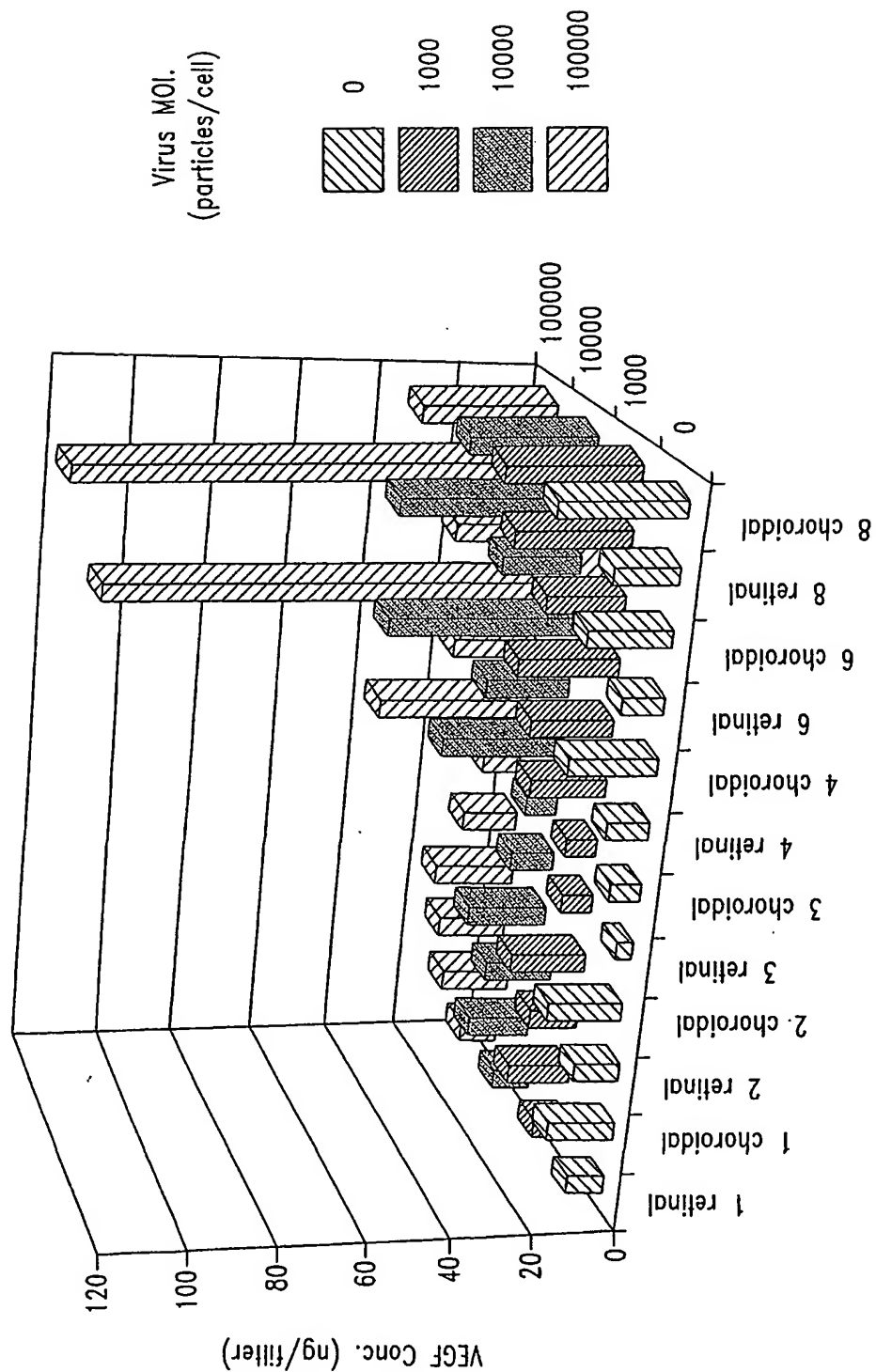


Fig. 23



Time after transfection (Day) and Polarity

Fig. 24

VEGF Secretion by hRPE After Infection with VEGF AV

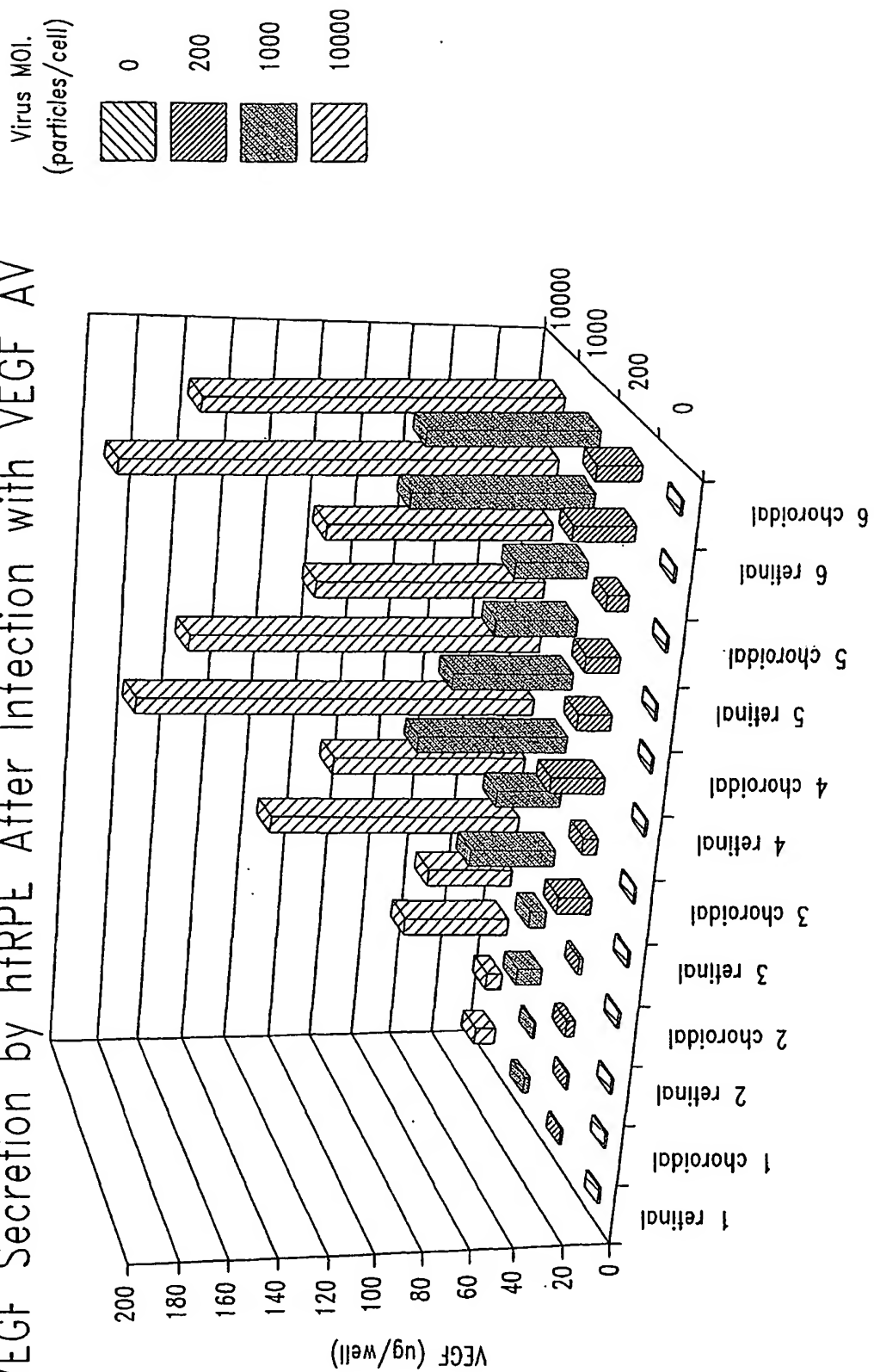


Fig. 25

Time after Infection (Day) and Polarity

Resistance of hRPE After Infection with VEGF AV

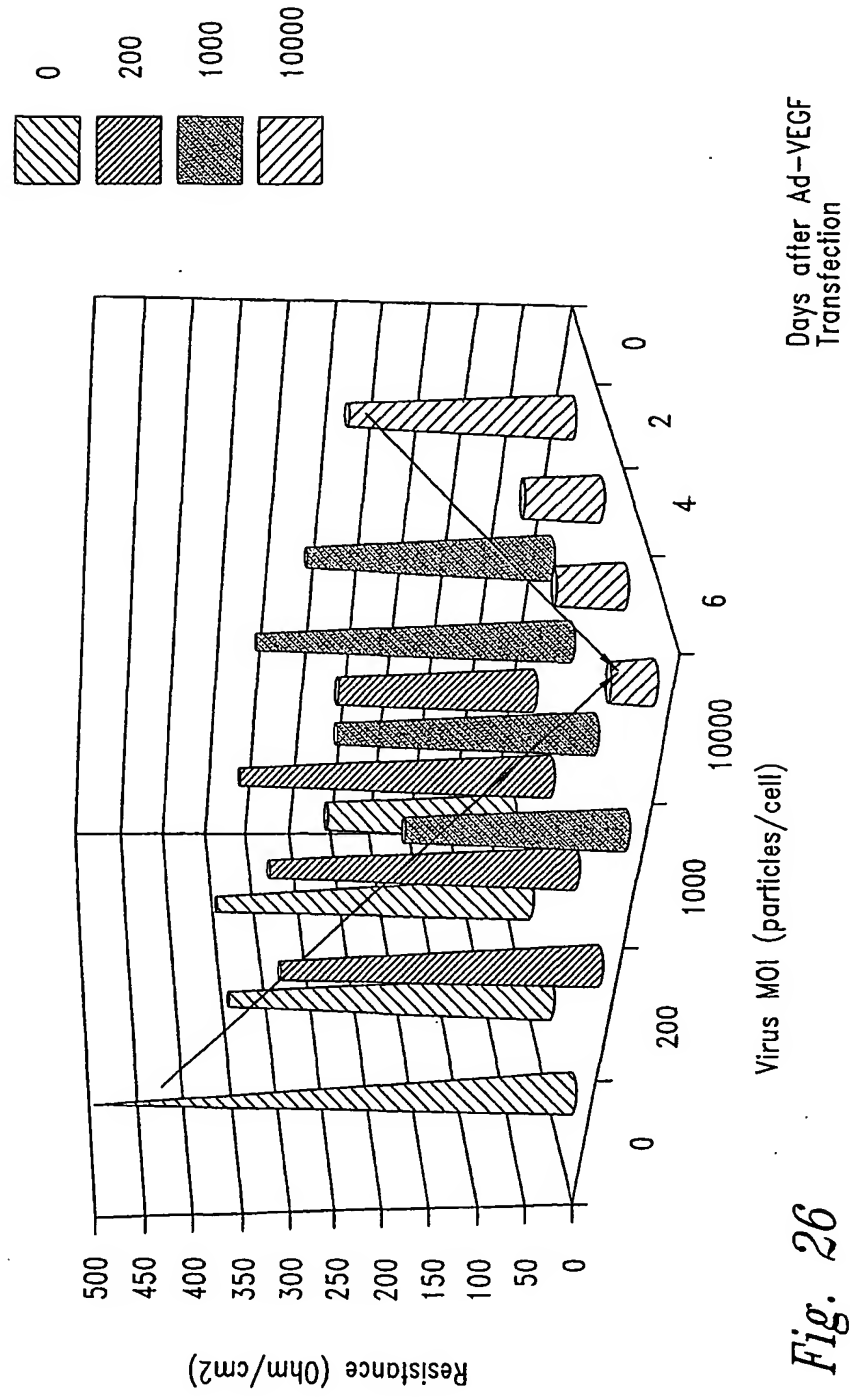


Fig. 26

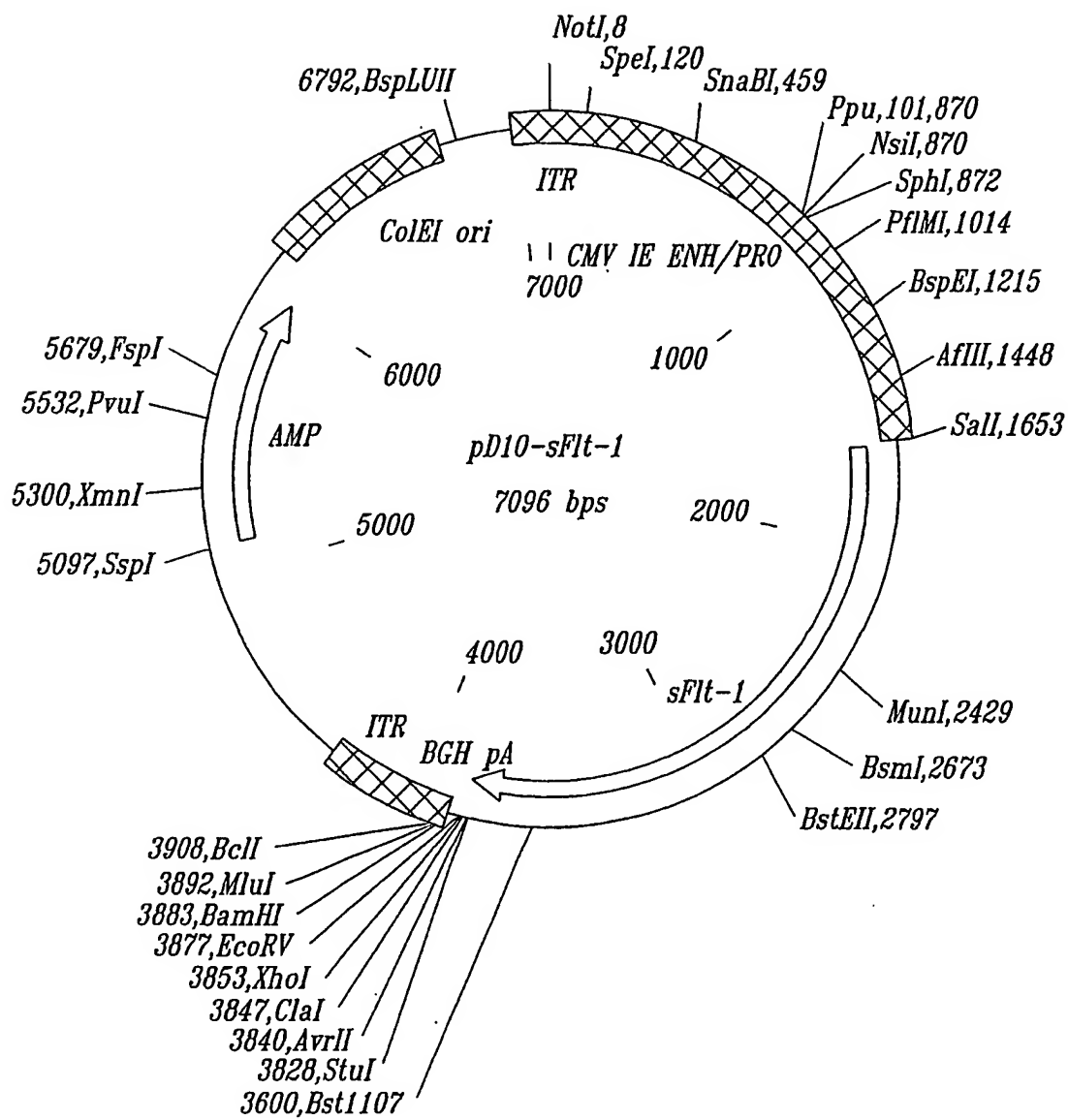


Fig. 27

Nucleotide Sequence of pD10-SF1t-1

AAACTTGGGCGCGGAATTCGACTCTAGGCCATTGCATACGTTGTATCTATATCATATATGTACATTTATATTGGCTCATGTCCAATATGACCGC
CATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGG
TAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGAC
GTCAATGGGTGGAGTATTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCGCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGT
TTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTGGCACAAA
ATCAACGGGACTTTCAAAATGTCTGAATAACCCGCCCCGTTGACGCAATGGGCGGTAGGCGGTACGGTGGGAGGTCATATAAGCAGAGCTCGTT
TAGTGAACCGTCAGATCGCTGGAGACGCCATCCAGCGTGTGTTGACCTCCATAGAAGACACCGGACCGATCCAGCTCCGCGGCCGGGAACGGTGCA
TTGGAACCGGATCCCCGTGCCAAGAGTGACGTAAGTACCGCTATAGACTCTATAGGCACACCCCTTGGCTCTTATGATGCTATAGTGTGTTTGG
CTTGGGGCTATACCCCCGCTCTTATGCTATAGGTGATGGTATAGCTTAGCCTATAGGTGGGTTATTGACCATATTGACCACTCCCCATTGG
TGACGATACTTTCATTACTAATCCATAACATGGCTCTTGGCACAATATCTCTATTGGCTATATGCCAATACTCTGTCTTCAGAGACTGACACGGA
CTCTGTATTTTACAGGATGGGGTCCATTATTTACAAATTCACATATACAACACGCGTCCCCGTGCCCGCAGTTTTATTAAACATAGCGTG
GGATCTCGACATCTCGGGTACGTGTTCCGGACATGGGCTCTTCCGGTAGCGGCGAGCTTCACATCCGAGCCCTGGTCCATCCGTCAGCGGCT
CATGGTCGCTCGGCAGCTCCTTGCTCTAACAGTGGAGGCCAGACTTAGGCACAGCACAATGCCACCACCACAGTGTGCGCACAAGGCGTGGCGG
TAGGGTATGTGTCTGAAAATGAGCTCGGAGATTGGGCTCGCACCTGGACGCAGATGGAAGACTTAAGGCAGCGGCAGAAGAAGATGCAGGCAGCTGAGT
TGTTGTATTCTGATAAGAGTCAGAGGTAATCCGTTGCGGTGCTGTTAACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGGC
CACCAGACATAATAGCTGACAGACTAACAGACTGTTCTTTCCATGGGTCTTTTCTGCAGTCACCGTCGTCGACCTAAGAATTGCGCTTTCACCATGG
TCAGTACTGGGACACCGGGTCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAGATCCTGAACTGA
GTTTAAAGGCCACCCAGCAGCATATGCAAGCAGGCCAGACTGCATCTCAATGCGGGGGAAGCAGCCATAAATGGTCTTGGCTGAATGGTGA
GTAAGGAAGCGAAAGGCTGAGCATAACTAATCTGCCTGTGGAAGAATGGCAACAATTTGTCAGTACTTTAACCTTGAACACAGCTCAAGCAAACC
ACACTGGCTTCTACAGCTGCAAAATCTAGCTGTACCTACTTCAAGAAGAAGGAACAGAATCTGCAATCTATATTTATTAGTGATACAGGTAGAC
CTTTCGTAGAGATGACAGTGAATCCCGAAATTATACACATGACTGAAGGAAGGAGCTCGTCATTCCCTGCGGGTTACGTACCTAACATCACTG
TTACTTTAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAGAACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAATGCAACGTACA
AAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCAATTTGTATAAGCAAACTATCTCACACATCGACAAACCAATAACATCATAGATGTCC
AAATAAGCACACCACCGCCAGTCAAATTACTTAGAGGCATACCTTGTCTCAATTGTACTGTCTACCACTCCCTTGAACACGAGAGTTCAAATGACCT
GGAGTTACCTGATGAAAAAATAAGAGAGCTTCGTAAGGCGAGCAATTGACCAAGCAATTCCTATGCCAACATATTTACAGTGTCTTACTATTG
ACAAAATGCAGAACAAAGACAAAGGACTTTATCTGTGCTGTAAGGAGTGGACCATCATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAG
CATTATCACTGTGAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGCTTACC GGCTCTCTATGAAAGTGAAGGCATTTCCCTCGC
CGGAAGTTGTATGGTTAAAGATGGGTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGACGTAAGTGAAG
AGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAAAGTCAATGTGTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAACCCAGATTT
ACGAAAAGGCGGTGTCATGTTTCCAGACCCGGCTCTTACCCTACTGGGACGAGACAAATCTGACTTGTACCGCATATGGTATCCCTCAACCTACAA
TCAAGTGGTTCTGGACCCCTGTAACCATATCATTCGAAGCAAGGTGTGACTTTTGTTCATAATGAAGAGTCTTTATCCTGGATGCTGACAGCA
ACATGGGAACAGAAATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAGAATAAGATGGCTAGCACCTTGGTTGTGGCTGACTCTAGAATTT
CTGGAATCTACATTTGATAGCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTGCCAATGGGTTTCATGTTAACT
TGGAAAAAATGCCGACGGAAGGAGAGGACCTGAACTGTCTTGACAGTTAACAAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAGTTA
ATAACAGAACAAATGCACTACAGTATTAGCAAGCAAAAAATGCCATCACTAAGGAGCACTCCATCACTCTTAATCTTACCATCATGAATGTTTCCCTGC
AAGATTGAGGACCTATGCTGAGAGCCAGGAATGTATACAGGGGAAGAAATCTCCAGAAGAAAGAAATTACAATCAGAGGTGAGCACTGCAACA
AAAAGGTGTTTTCTCTGGATCTCCAAATTTAAAGACAGGAATGATTGTACCACAAAGTAATGTAAACATTAAAGGACTCATTAAAGGTAA
CAGTTGTCTCATATCATCTTGATTTATTGTCACTGTTGCTAACCTTCAGGCTCAAGGGCAATTCAGGCCTAAGCTTCTAGGTATCGATCTCGAGCAA
GTCTAGAAAGCCATGGATATCGGATCCACTACGCGTTAGAGCTCGCTGATCAGCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTTGTTGCCCTC

Fig. 28A

CCCCGTGCCCTTCTTGACCCCTGGAAGGTGCCACTCCACTGTCTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTAT
TCTGGGGGGTGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGGGGGTGGGCGAAGAACTCCAGCATGAGATCCCGCGCTGGA
GGATCATCCAGCTAGCAAGTCCCATCAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCGCCGA
CGCCCGGGCTTTGCCCCGGGGGCTCAGTGAGCGAGCGCGCCAGCGATTCTCTTGTGTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGT
AGAGACCTCTCAAAAATAGCTACCTCTCCGGCATGAATTTATCAGCTAGAACGGTTGAATATCATATTGATGGTGATTGACTGTCTCCGGCCTTTCT
CACCCGTTTGAATCTTTACCTACACATTACTCAGGCATTGCATTTAAATATATGAGGGTTCTAAAAATTTTATCCTTGCGTTGAAATAAAGGCTTCT
CCCGCAAAAGTATTACAGGGTCATAATGTTTTTGGTACAACCGATTAGCTTTATGCTCTGAGGCTTATTGCTTAATTTTGCTAATCTTTGCTTGC
CTGTATGATTTATTGGATGTTGGAATTCCTGATGCGGTATTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCACTCTCAGTACAAT
CTGCTCTGATCGCGCATAGTTAAGCCAGCCCCGACACCGCCAAACACCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACA
AGCTGTGACCGCTCTCCGGAGCTGCATGTGTGAGAGTTTCCCGTCATACCGAAACGCGGAGACGAAAGGGCTCGTGATACGCTATTTTATA
GGTTAATGTCAAGATAAATAGTTTCTTAGAGCTCAGGTGGCACTTTTGGGGAAATGTGCGCGGAACCCCTATTTGTTATTTTCTAAATACATTC
AAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATCAACATTTCCGTGTCGCCCTTAT
TCCCTTTTTCGGGCAATTTTGCTTCTGCTTCTGCTCAGCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGACGAGTGGGTTA
CATCGAAGTGGATCTCAACAGCGGTAAAGTCTTGAGAGTTTTGCGCCCCAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGC
GGTATTATCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAAATGACTTGGTTGAGTACTCACCAGTCACAGAAAGCA
TCTTACGGATGCGATGACAGTAAGAGAATTATGCAAGTGTGCATAAACCATGAGTGATAACACTGCGGCCAAGTTACTCTGACAACGATCGGAGGACC
GAAGGAGCTAACCGCTTTTTCACAAATGCGGGATCATGTAACCTGCGCTTGATCGTTGGGAACCGAGCTGAATGAAGCCATACCAAAACGACGAGCG
TGACACCAGATGCTGTAGCAATGGCAACAAGTTGCGCAAACTATTAAGTGGCAACTACTTACTTAGCTTCCCGGCAACAATTAAGATGAGAT
GGAGGCGGATAAAGTTGAGGACCACTTCTGCGCTCGGCCCTCCGGCTGGCTGGTTATTGCTGATAAATCTGGAGCGGTGAGCGTGGGTCTCGCGG
TATCATTGCAAGCTGGGGCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACAGCAGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGAT
CGCTGAGATAGGTGCTCACTGATTAAAGCATTGGTAAGTGTGAGCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTAAATTTAA
AAGGATCTAGGTGAAGATCCTTTTGTATAATCTCATGACCAAAATCCCTTAAAGTGAGTTTTGCTTCACTGAGCGTCAGACCCCGTAGAAAAGATCAA
AGGATCTTCTTGAGATCCTTTTTCGCGCGTAATCTGCTGCTTGCAACAAAAAACACCGCTACCAGCGGTGGTTGTGTTGCGGATCAAGAGCT
ACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAACTACTGCTTCTAGTGAGCGGTAGTTAGGCCACCCTTCAAGAACTC
TGAGCAGCGCTACATACTCGCTCTGCTAATCTGTTACCAAGTGGTGTGCGAGTGCGATAAGTGTGCTTACCGGGTTGACTCAAGACGATA
GTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGTTGCTGCACACAGCCAGCTTGAGCGGAACGACCTACACCGAACTGAGATACCTACAGCG
TGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGACAGGTATCCGTTAAGCGGACGGGTCGGAACAGGAGAGCGACGAGGGAGCTTCC
AGGGGGAACGCTGGTATCTTTATAGTCTGTGCGGTTTCCGCACCTCTGACTTGAGCGTGGATTTTGTGATGCTCGTCAGGGGGGCGAGCCTATG
GAAAAACGCCAGCAACGCGGCTTTTACGGTTCTGCGCTTTTGTGCGCTTTTGTCTCACATGTTCTTCTGCGTTATCCCTGATTCTGTGGATAA
CCGTATTACCGCTTTGAGTGAGCTGATACCGCTCGCCGACGCGAAGCAGCGAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACG
CAAAACCGCTCTCCCGCGGTTGGCCGATTCTTAATGCAGCTGGCGCGCTCGCTCGCTCACTGAGGCGCGCGGCAAGCCCGGCGTGGGGCGAC
CTTTGGTCGCGCGGCTCAGTGAGCGAGCGAGCGCGAGAGGGAGTGGCCAACTCCATCACTGAT

Fig. 28B

HumanFGF-20

atggctcccttagccgaagtcggggccttctggcgccctggagggttgggccagcag
M A P L A E V G G F L G G L E G L G Q Q

gtgggttcgcatttctgttgctcctgccggggagcggccgcgctgtggcgagcgc
V G S H F L L P P A G E R P P L L G E R

aggagcgcggcggagcggagcgcgcggcgccggggcgtgcgcagctggcgacctg
R S A A E R S A R G G P G A A Q L A H L

cacggcatcctgcgcggcggcagctctattgccgcaccggcttccacctgcagatcctg
H G I L R R R Q L Y C R T G F H L Q I L

cccgcggcagcgtgcagggcaccggcaggaccacagccttctcggtatcttggaattc
P D G S V Q G T R Q D H S L F G I L E F

atcagtgtggcagtgaggctgtcagtaggtgtggacagtggtctctatcttggg
I S V A V G L V S I R G V D S G L Y L G

atgaatgacaaaggagaactctatggatcagagaaacttactccgaatgcattttagg
M N D K G E L Y G S E K L T S E C I F R

gagcagtttgaagagaactggtataacacctattcatctaacaatataaacaatggagac
E Q F E E N W Y N T Y S S N I Y K H G D

actggccgcaggtatcttggcacttaacaaagcggaaactccaagagatggcgccagg
T G R R Y F V A L N K D G T P R D G A R

tccaagaggcatcagaaatttacacatttcttacctagaccagtggtccagaaagagtt
S K R H Q K F T H F L P R P V D P E R V

ccagaattgtacaaggacactactgatgtacacttga
P E L Y K D L L M Y T

Fig. 29

Mouse FGF-21 cDNA in pGEM-T

gagcgcagccctgatggaatggatgagatctagagttgggaccctgggactgtgggtccg SEQ ID NO: 1
 M E W M R S R V G T L G L W V R SEQ ID NO: 2

actgctgctggctgtcttctgctggtgggtctaccaagcataccccatccctgactccag
L L L A V F L L G V Y Q A Y P I P D S S

ccccctcctccagtttgggggtcaagtccggcagaggtacctctacacagatgacgacca
P L L Q F G G Q V R Q R Y L Y T D D D Q

agacactgaagccacctggagatcaggaggatggaacagtggtaggcgcagcacaccg
D T E A H L E I R E D G T V V G A A H R

cagtcagaaagtctcctggagctcaaagccttgaagccaggggtcattcaaatcctggg
S P E S L L E L K A L K P G V I Q I L G

tgtcaaagcctctaggtttcttgcacacagccagatggagctctctatggatcgctca
V K A S R F L C Q Q P D G A L Y G S P H

ctttgatcctgaggcctgcagcttcagagaactgctgctggaggacggttacaatgtga
F D P E A C S F R E L L L E D G Y N V Y

ccagtcctgaagcccatggcctgcccctgcgtctgcctcagaaggactcccaaaccagga
Q S E A H G L P L R L P Q K D S P N Q D

tgcaacatcctggggacctgtgcgcttctgcccattgccaggcctgctccacgagcccca
A T S W G P V R F L P M P G L L H E P Q

agaccaagcaggattcctgccccagagccccagatgtgggtcctctgacccccctgag
D Q A G F L P P E P P D V G S S D P L S

catggtagagcctttacagggccgaagccccagctatgcgtcctgactcttctgaatc
M V E P L Q G R S P S Y A S

Fig. 30

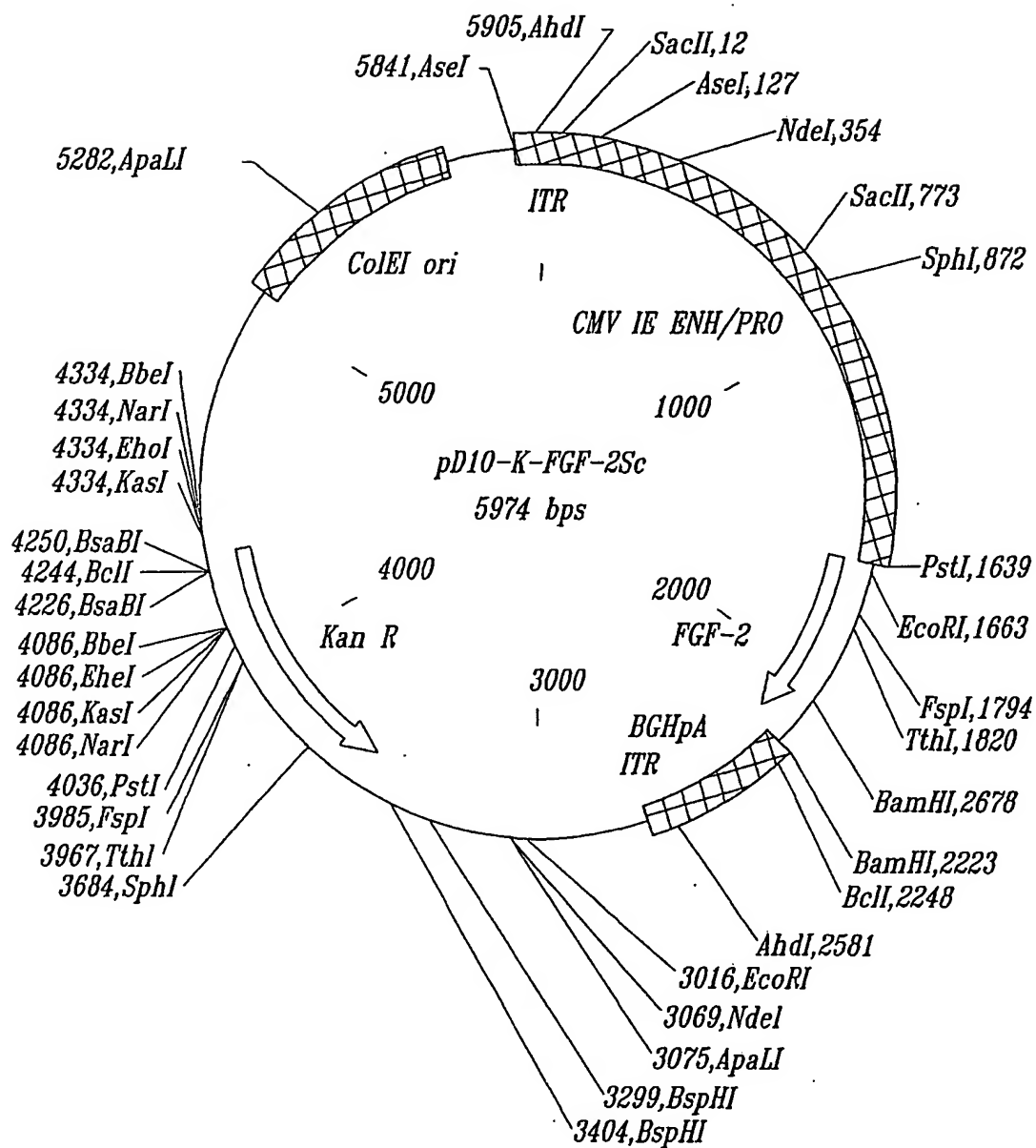


Fig. 31

AAAACCTGCGGCCGCGGAATTCGACTCTAGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACC
GCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTT
ACGGTAAATGGCCGCTGGCTGACCGCCCAACGACCCCGCCCATAGAGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCGCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG
GTGATGCGGTTTTGGCAGTACACCAATGGCGGTGGATAGCGGTTTGACTCACGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
TTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCCGCCCGTTGACGCAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATAT
AAGCAGAGCTCGTTTAGTGAACGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGGGACCGATCCAGCTCCGC
GGCCGGGAACGGTGCATTGGAACGCGGATTCCCGTGCCAGAGTGACGTAAGTACCGCTATAGACTCTATAGGCACACCCCTTTGGCTCTTATGC
ATGCTATACTGTTTTGGCTTGGGGCTATACACCCCGCTCTTATGCTATAGGTGATGGTATAGCTTAGCTATAGGTGGGTATTGACCATT
ATTGACCACTCCCTATTGGTGACGATACTTTCCATTACTAATCCATAACATGGCTCTTGGCACAACATCTCTATTGGCTATATGCCAATACTCT
GTCTTCAGAGACTGACACGGACTCTGTATTTTACAGGATGGGTCCATTTATTATTACAAATTCACATATACAACAACGCGTCCCGTGCCC
GCAGTTTTTATTAACATAGCGTGGGATCTCCGACATCTCGGGTACGTGTTCCGGACATGGGCTCTTCTCCGGTAGCGCGGAGCTTCACATCCGA
GCCCTGGTCCCATCCGTCCAGCGGCTCATGGTGGTCCGCGAGCTCTTGCTCCTAACAGTGGAGGCCAGACTTAGGCACAGCAATGCCACCACC
ACCAGTGTGCGCACAAGGCGGTGGCGGTAGGGTATGTGTCTGAAATGAGCTCGGAGATTGGGCTCGCACCTGGACGCAGATGGAAGACTTAAGGC
AGCGGCAGAAGAAGATGCAGGCAGCTGAGTTGTTGTAATCTGATAAGAGTCAAGGTAACCTCCGTTGCGGTGCTGTTAACGGTGGAGGGCAGTGTA
GTCTGAGCAGTACTCGTTGCTGCGCGCGCGCCACCAGACATAATAGCTGACAGACTAACAGACTGTTCTTCCATGGGTCTTTCTGCACTCACC
GTCTGACCTAAGAATTAGGTATGGCTGCTGTTCTATCACTACCTGCCAGCTCTGCCAGAAGACGGTGGTCTGGTGGCTTCCACCAGGTCA
CTTCAAAGACCCAAACGCTCTGTACTGCAAAAACGGTGGTTTCTCTGCGCATCCACCCGACGCGCGAGTGGACGGGGTCCGCGAGAAGAGCGAC
CCACACATCAAACTACAATTCAGCAGAAGAGAGAGGGGTTGTCTATCAAGGAGTGTGTGCAAACCGTTACCTTGCTATGAAAGAAGATGGAA
GATTACTAGCTTCTAAATGTGTTACAGACGAGTGTCTTTTTTGAACGATTGGAGTCTAATAACTACAATACTTACCGGTCAAGGAAATACACCAG
TTGGTATGTGGCACTGAAACGAACGGGCAGTATAAACTTGGATCCAAAACAGGACCTGGGCAGAAAGCTATACTTTTCTTCCAATGTCTGCTAAG
AGCTGATCTTAATGGCAGCATCTGATCTCATTTTACATGAAGCTTCTAGGTATCGATCTCGAGCAAGTCTAGAAAGCCATGGATATCGGATCCACT
ACGCGTTAGAGCTCGTGCATCAGCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCGTGCTTCTTGACCTTGAAGGT
GCCACTCCCACTGTCTTTCTTAATAAAATGAGGAAATGCAATCGCATGTCTGAGTGGTGTCTTCTATTCTGGGGGGTGGGGTGGGGCAGGACA
GCAAGGGGGAGGATTGGGAAGACAATAGCAGGGGGTGGGCGAAGAACTCCAGCATGAGATCCCGCGCTGGAGGATCATCCAGCTAGCAAGTCCCA
TCAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCGGGCGACCAAGGTCGCCGACGCGCGGGCTTTGCGCGGGCGG
CCTCAGTGAGCGAGCGGCGGCCAGCGATTCTCTGTTTGTCTCAGACTCTCAGGCAATGACCTGATAGCTTTGTAGAGACCTCTCAAAAATAGC
TACCCTCTCCGGCATGAATTTATCAGCTAGAACGGTTGAATATCATATTGATGGTGAATTTGACTGTCTCCGCGCTTCTCACCGGTTTGAATCTTTA
CCTACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCAAAAGTATTAC
AGGGTCATAATGTTTTTGGTACAACCGATTAGCTTTATGCTCTGAGGCTTTATTGCTAATTTTGTAAATCTTTGCTTGGCTGTATGATTATT
GGATGTTGGAATTCCTGATGCGGTATTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCACCTCTCAGTACAATC

Fig. 32A

TGCTCTGATGCCGCATAGTTAAGCCAGCCCGACACCCGCCAACACCCGCTGACGGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC
AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGACAGGTTTTACCGTCATCACCAGAACGCGCGAGACGAAAGGGCTCGTGATACGCCATTTTT
ATAGGTTAATGTGATGATAAATGGTTTCTTAGACGTGAGGTGGCATTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATA
CATTCAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATGTACCGTCAAGAAGGCGATAGAAGGCGATGCGCTGCCAATC
GGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTGAGCCATTGCTTCAGCAATATCAGGGTAGCCAACGCTATGTCTGATAGCGGTCCGCCA
CACCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGGGTACGACGAGATCCTC
GCGCTCGGGCATGCGCGCTTGAGCCTGGCGAACAGTTGCGCTGGCGCGAGCCCTGATGCTTTCGTCCAGATCATCTGATCGACAAGACCGGCT
TCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGTGCGAATGGGCAGGTAGCCGATCAAGCGTATGACGCCGCCGATTGATCAG
CCATGATGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCGGCACCTTCGCCAATAGCAGCCAGTCCCTCCCGCTTCACT
GACAACGTGAGCACAGTGCAGCAAGGAACGCCGCTGCTGGCCAGCCACGATAGCCGCGCTGCTCGTCTGCAAGTTCATTGAGGGCACCAGGACAGG
TCGGTCTTGACAAAAGAACCGGGCGCCCTGCGCTGACAGCCGGAACAGCGGCGCATCAGAGCAGCGATTGTCTGTTGCGCCAGTCATAGCCGA
ATAGCCTCTCCACCCAAGCGCGGAGAACCTGCGTGCAATCCATCTGTTCAATCATGCGAAACGATCCTCATCTGTCTCTTGATCAGATCTTGA
TCCCCTGCGCCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGACAGGCTTCCCAACCTTACCAGAGGGCGCCCGAGCTGGCAATTC
GGTTCGCTTGCTGTCCATAAAACCGCCAGTCTAGCTATCGCCATGTAAGCCCACTGCAAGTACCTGCTTTCTCTTTGCGCTTGGTTCCTCTG
TCCAGATAGCCAGTAGCTGACATTCATCCGGGTGACGACCGTTTCGCGGATGGCTTTCTACGTGTTCCGCTTCTTTAGCAGCCCTTGGCGCC
TGAGTGCTTGCGCGCAGCGTAAGCTGTCAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCT
TATAAATCAAAAGAATAGCCGAGATAGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGCG
GAAAAACCGTCTATCAGGCGATGGCGGATCAGCTTATGCGGTGTAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTCCG
CTTCTCGCTCACTGACTCGCTGCGCTCGGTGTTGCGCTGCGCGAGCGGTATCAGCTCAAGGCGGTAAATACGGTTATCCACAGAATCAGG
GGATAACGAGGAAAGACATGCGCGCGCCACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCGGTTTTTCC
ATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCC
TGGAGGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGCGCTTCTCATAGC
TCACGCTGTAGGTATCTCAGTTGCGGTGATGTTGCTCCAGCTGGGCTGTGTGACGAAACCCCGTTACGCCGACCGCTGCGCTTATCCG
GTAATATCGTCTTGAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGG
TGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAA
AGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGCGTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCA
AGAAGATCCTTTGATCTTTTCTTACTGAACGGTGATCCCAACCGGAATTGCGGCCATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAACCG
TATTACGCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGC
AAACCGCTCTCCCGCGCGTTGGCGGATTCAATATGACAGCTGGCGCGCTGCTGCTCACTGAGGCGCCCGGGCAAGCCCGGCGCTGGGGCA
CCTTTGTCGCGCGGCTCAGTGAGCGAGCGAGCGCAGAGGGAGTGCCAACTCCATCACTGAT

Fig. 32B

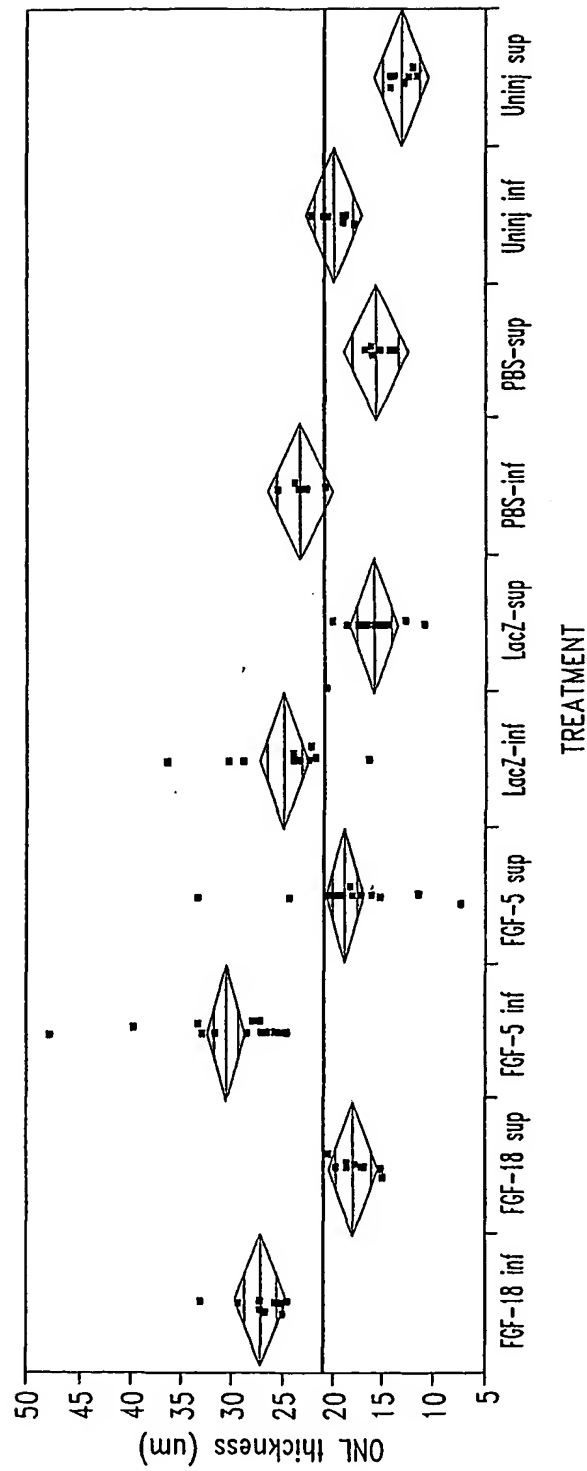
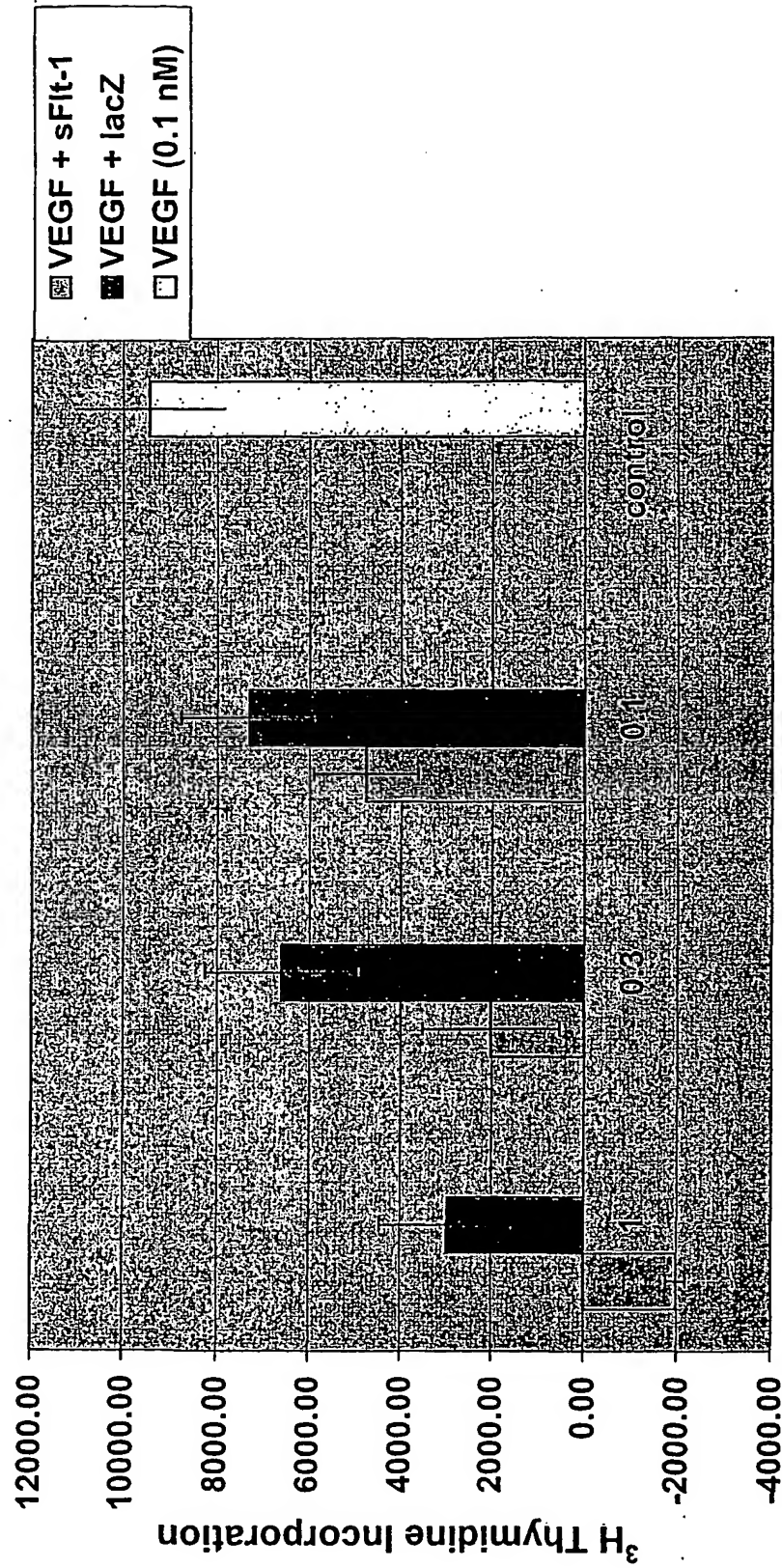


Fig. 33

Inhibition of HMVEC Proliferation by sFlt-1 rAAV



sFlt-1 Protein in Conditioned Media (in nM)

FIGURE 34



Figure 35. Fluorescein Angiography

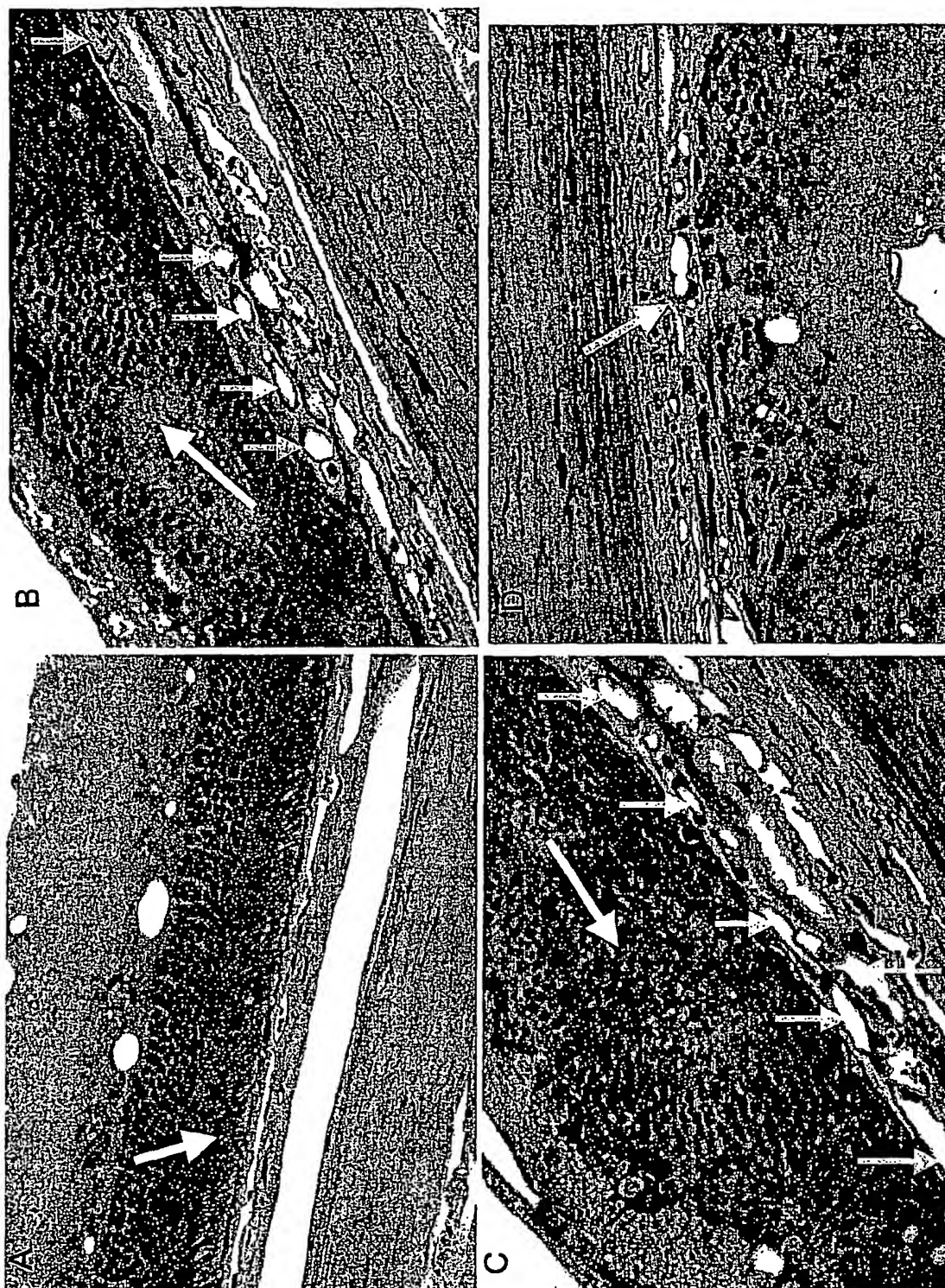


Figure 36. Epoxy Sections

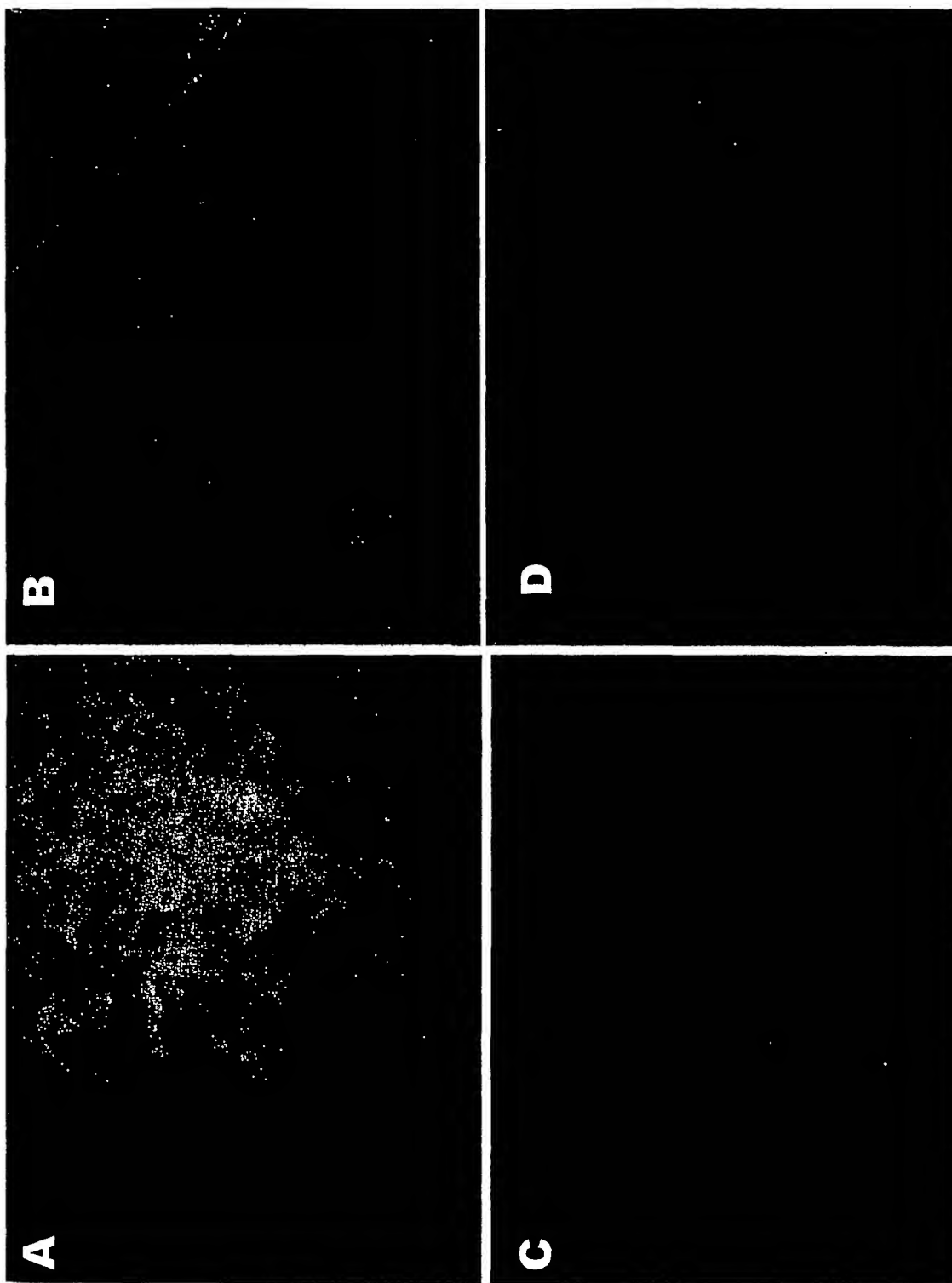
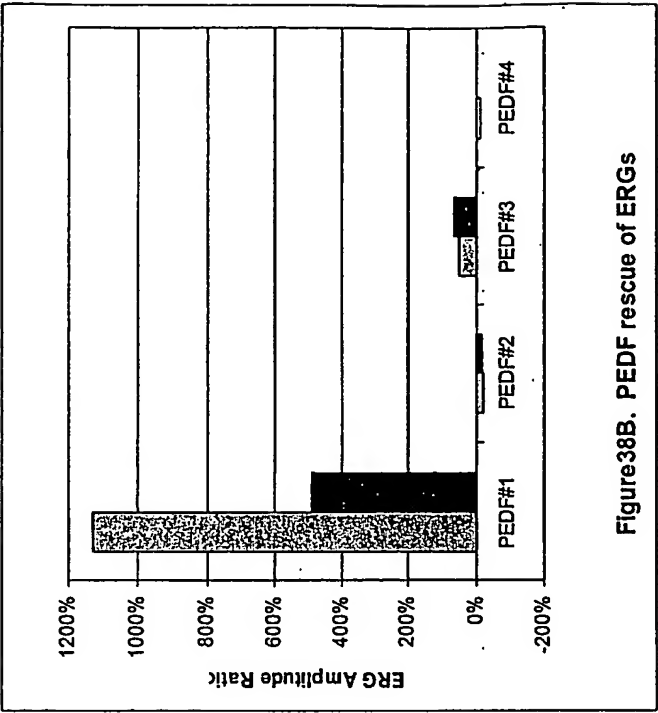
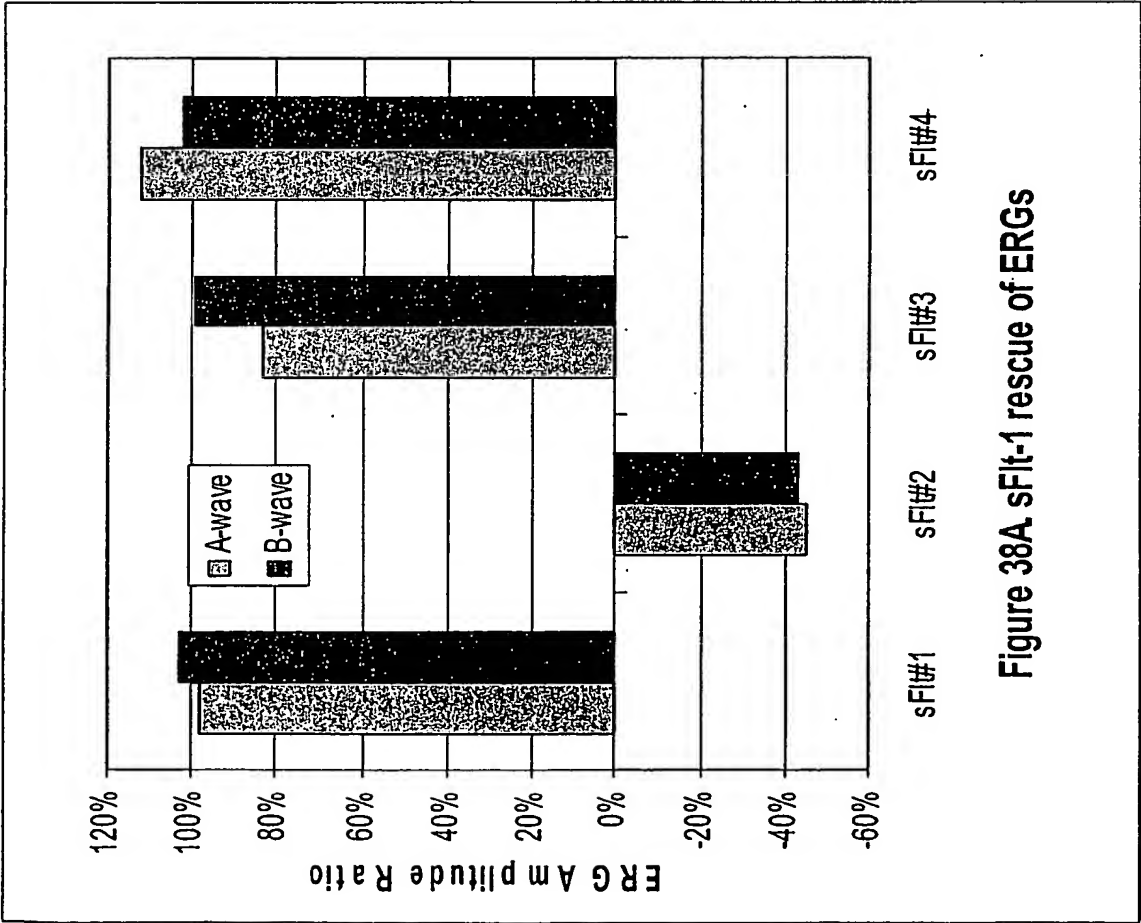


Figure 37. Lectin and BrdU staining



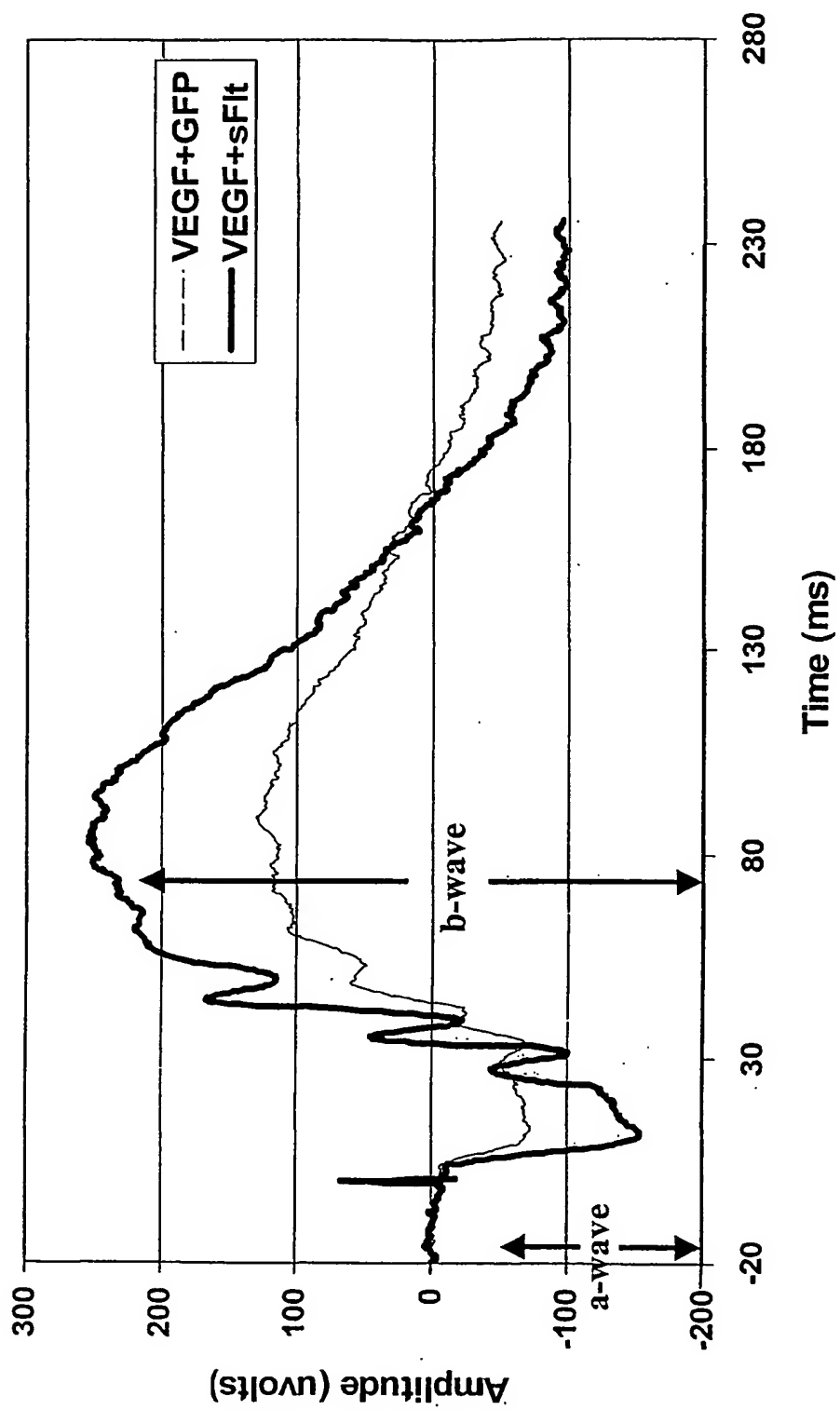


Figure 39. ERG of 070900 Rat#4 on 082300 (6 wk)

SEQUENCE LISTING

<110> Manning, William C., Jr.
 Dwarki, Varavani J.
 R ndahl, Katherine
 Zhou, Shang-Zhen
 McGee, Laura H.
 Lau, Dana
 Flannery, John G.
 Miller, Sheldon
 Wang, Fei
 Di Polo, Adriana

<120> USE OF RECOMBINANT GENE DELIVERY VECTORS
 FOR TREATING OR PREVENTING DISEASES OF THE EYE

<130> PP01588.005 (20263.40)

<140> US/09,665,493

<141> 2000-09-20

<160> 12

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 6514

<212> DNA

<213> Homo sapien

<400> 1

accatgtagc	ggccctgcgc	gctcgcgcgc	tcactgaggc	cgccccgggca	aag�ccccgggc	60
gtcggg�gcac	ctttgggcgc	ccggcctcag	tgagcgcgcg	agcgcgcaga	gagggagtg	120
ccaa�tccat	cactaggggt	tccttgtagt	taatgattaa	cccgccatgc	tacttatcta	180
cgtagccatg	ctctagggaa	ttggccgcgc	aatttcgact	ctaggccatt	gcatacgttg	240
tatctatc	ataatatgta	catttatatt	ggctcatgtc	caatatgacc	gccatgttga	300
cattgattat	tgactagtta	ttaatagtaa	tcaattacgc	ggtcattagt	tcatagccca	360
tatatggagt	tccgcgttac	ataacttacg	gtaaatggcc	cgcc�ggctg	accg������	420
gac�������	cattgacgtc	aataatgacg	tatgtt����	tagtaacgcc	aatagggact	480
ttccattgac	gtcaatgggt	ggagtattta	cggtaaa�tg	cccacttggc	agtacatcaa	540
gtgtatcata	tgccaagtcc	g��������	gacgtcaatg	acggtaaatg	g��������g	600
cattatg���	agtacatgac	cttacgggac	ttt�������	ggcagtagac	����������	660
gtcatcgcta	ttaccatggg	gatgcggttt	tggcagtaca	ccaatgggcg	tgatagcgcg	720
tttgactcac	ggggatttcc	aagt������	cccattgacg	tcaatgggag	tttgttttgg	780
cac�������	aacgggactt	t��������g	cgtaataa��	ccg�������	gacg�����g	840
ggcggtaggc	gtgtacgggt	ggagg����	ataagcagag	ctcgtttagt	gaaccgtcag	900
atcg������	gacg������	acg�������	gac�������	gaagacacgc	ggaccgatcc	960
ag��������	g��������g	gtgcattgga	acgcggattc	cccgtg����	gagt������	1020
agtac�����	atagact���	taggcacac��	c���������	ttatgcatgc	tata������	1080
ttgg������	g���������	cc��������	tatg������	gtgatggtat	ag��������	1140
atagg�����	gttattgac��	attattgac��	act�������	tggtgacgat	act��������	1200
actaat����	aacatgg���	tttg������	ctat������	tggtat����	ccaat������	1260
gtc�������	gactgacacg	gact������	ttttacagga	tggg������	ttattattta	1320
caaattcaca	tataca����	cgcg������	cgtg������	gtttttatta	aacatagcgt	1380

actaatccat	aacatggctc	tttgccacaa	ctatctctat	tggctatatg	ccaatactct	1260
gtccttcaga	gactgacacg	gactctgtat	ttttacagga	tgggggtccat	ttattattta	1320
caaattcaca	tatacaacaa	cgccgtcccc	cgtgcccgca	gtttttatta	aacatagcgt	1380
gggatctccg	acatctcggg	tacgtgttcc	ggacatgggc	tcttctccgg	tagcggcgga	1440
gcttccacat	ccgagccctg	gtcccatccg	tccagcggct	catggctcgt	cggcagctcc	1500
ttgctcctaa	cagtggaggc	cagacttagg	cacagcacia	tgcccaaccac	caccagtgtg	1560
ccgcacaagg	ccgtggcggg	agggtatgtg	tctgaaaatg	agctcggaga	ttgggctcgc	1620
acctggacgc	agatggaaga	cttaaggcag	cggcagaaga	agatgcaggc	agctgagttg	1680
ttgtattctg	ataagagtca	gaggtaactc	ccgttgccgg	gctgttaacg	gtggagggca	1740
gtgtagctcg	aaacagtactc	gttgctgccc	cgcgcgccac	cagacataat	agctgcagaa	1800
ctaacagact	gttcctttcc	atgggtcttt	tctgcagtca	ccgtcgtcga	cctaagaatt	1860
caggcctaag	cttcctaggt	atcgatctcg	agcaagtcta	gagggagacc	acaacggttt	1920
ccctctagcg	ggatcaatcc	cgcccccccc	cctaacgtta	ctggccgaag	ccgcttgga	1980
taaggccggg	gtgctgttgt	ctatatgtta	ttttccacca	tattgccgtc	ttttggcaat	2040
gtgagggccc	ggaaacctgg	ccctgtcttc	ttgacgagca	ttcctagggg	tctttccctt	2100
ctcgccaaag	gaatgcaagg	tctgttgaat	gtcgtgaagg	aagcagttcc	tctggaagct	2160
tcttaaggac	aaacaacgtc	tgtagcgacc	ctttgcaggc	agcggaaacc	cccactggc	2220
gacaggtgcc	tctgcggcca	aaagccaagt	gtataagata	cacctgcaaa	ggcggcacia	2280
ccccagtgcc	acgttgtgag	ttggatagtt	gtggaaagag	tcaaattggc	ctcctcaagc	2340
gtattcaaca	aggggctgaa	ggatgcccag	aaggtaaccc	attgtatggg	atctgatctg	2400
gggcctcggg	gcacatgctt	tacatgtgtt	tagtcgaggt	taaaaaaacg	tctaggcccc	2460
ccgaaccacg	gggacgtggg	tttcctttga	aaaacacgat	aataccatgg	ccgcggggag	2520
catcaccacg	ctccagccc	tgccggagga	cggcggcagc	ggcgctttcc	cgcggggcca	2580
cttcaaggac	cccaagcggc	tgtactgcaa	gaacgggggc	ttcttctctg	gcacccaacc	2640
cgacggccga	gtggacgggg	tccgcgagaa	gagcgcacca	cacatcaaac	tacaacttca	2700
agcagaagag	agaggggttg	tgtctatcaa	aggagtgtgt	gcaaaccggt	accttgctat	2760
gaaagaagat	ggaagattac	tagcttctaa	atgtgttaca	gacgagtgtt	tcttttttga	2820
acgattggag	tctaataact	acaatactta	ccggtcaagg	aaatacacca	gttggtatgt	2880
ggcactgaaa	cgaactgggc	agtataaact	tggatccaaa	acaggacctg	ggcagaaagc	2940
tatacttttt	cttccaatgt	ctgctaagag	ctgatcttaa	tggcagcatc	tgatctcatt	3000
ttacatgaag	ctggctggcat	ccctgtgacc	cctccccagt	gcctctctctg	gccctggaag	3060
ttgccactcc	agtgcccacc	agccttgtcc	taataaaatt	aagttgcac	atcttctctg	3120
actaggtgtc	cttctataat	attatggggg	ggaggggggt	ggatgggagc	aaggggcaag	3180
ttgggaagac	aacctgtagg	gcctgcgggg	tctattggga	accaagctgg	agtgacgtgg	3240
cacaatcttg	gtcactgca	atctccgcct	cctgggttca	agcgattctc	ctgcctcagc	3300
ctcccgagtt	gttgggattc	caggcatgca	tgaccaggct	cagctaattt	ttgttttttt	3360
ggtagagacg	gggtttcacc	atattggcca	ggctggtctc	caactcctaa	tctcaggtga	3420
tctaccaccc	ttggcctccc	aaattgtctg	gattacaggc	gtgaaccact	gctcccttcc	3480
ctgtccttct	gatttttaaa	taactatacc	agcaggagga	cgtccagaca	cagcataggc	3540
tacctggcca	tgcccaaccg	gtgggacatt	tgagttgctt	gcttggcact	gtcctctcat	3600
gcgttgggtc	cactcagtag	atgcctgttg	aattatcgga	tccactacgc	gttagagctc	3660
gctgatcagc	ctcgactgtg	ccttctagtt	gccagccatc	tgttgtttgc	ccctcccccg	3720
tgcttctctt	gaccctggaa	ggtgccactc	ccactgtcct	ttcctaataa	aatgaggaaa	3780
ttgcatacgca	ttgtctgagt	agggtgtcatt	ctattctggg	gggtgggggtg	gggcaggaca	3840
gcaagggggg	ggattgggaa	gacaatagca	ggggggtggg	cgaagaactc	cagcatgaga	3900
tccccgcgct	ggaggatcat	ccagccaatt	ccctagagca	tggctacgta	gataagtagc	3960
atggcggggt	aatcattaac	tacaaggaa	ccctagtgat	ggagttggcc	actccctctc	4020
tgcgcgctcg	ctcgctcact	gaggccgggc	gaccaaagg	cgcgcgacgc	ccgggctttg	4080
ccggggcggc	ctcagtgagc	gagcagagcg	gcaggggggtg	ggcgaagaac	tccagcatga	4140
gatccccgcg	ctggaggatc	atccagccgg	cgtccccgaa	aacgattccg	aagcccaacc	4200
tttcatagaa	ggcggtcggtg	gaatcgaaat	ctcgtgatgg	caggttgggc	gtcgcttggt	4260
cgggtcatttc	gaaccccaga	gtcccgctca	gaagaactcg	tcaagaaggc	gataagaagg	4320
gatgcgctgc	gaatcgggag	cggcgatacc	gtaaagcacg	aggaagcggt	cagcccatcc	4380
gccgccaagc	tcttcagcaa	tatcacgggt	agccaacgct	atgtcctgat	agcggctccg	4440

cacacccagc	cggccacagt	cgatgaatcc	agaaaagcgg	ccatthttcca	ccatgatatt	4500
cggcaagcag	gcatcgccat	gggtcacgac	gagatcctcg	ccgtcgggca	tgcgcgcctt	4560
gagcctggcg	aacagttcgg	ctggcgcgag	cccctgatgc	tcttcgtcca	gatcatcctg	4620
atcgacaaga	ccggcttcca	tccgagtagc	tgctcgctcg	atgcgatgtt	tcgcttggtg	4680
gtcgaatggg	caggtagccg	gatcaagcgt	atgcagccgc	cgcattgcat	cagccatgat	4740
ggatactttc	tcggcaggag	caagggtgaga	tgacaggaga	tcttgccccg	gcacttcgcc	4800
caatagcagc	cagtcccttc	ccgcttcagt	gacaacgtcg	agcacagctg	cgcaaggaaac	4860
gcccgtcgctg	gccagccacg	atagccgcgc	tgcctcgctcc	tgcagttcat	tcagggcacc	4920
ggacaggtcg	gtcttgacaa	aaagaaccgg	gcgcccctgc	gctgacagcc	ggaacacggc	4980
ggcatcgagc	cagccgattg	tctgttggtc	ccagtcatag	ccgaatagcc	tctccacca	5040
agcggccgga	gaacctgcgt	gcaatccatc	ttgttcaatc	atgcgaaacg	atcctcatcc	5100
tgtctcttga	tcagatcttg	atccccgtcg	ccatcagatc	cttggcggca	agaaagccat	5160
ccagtttact	ttgcagggct	tcccaacctt	accagagggc	gccccagctg	gcaattccgg	5220
ttcgcttgct	gtccataaaa	ccgcccagtc	tagctatcgc	catgtaagcc	cactgcaagc	5280
tacctgcttt	ctctttgcgc	ttgcggtttc	ccttgctccag	atagcccagt	agctgacatt	5340
catccggggg	cagcaccggt	tctgcggact	ggctttctac	gtgttcgcgt	tccttttagca	5400
gccccttgccg	cctgagtgct	tgccgcagcg	tgaagctgtc	aattccgcgt	taaatttttg	5460
ttaaatcagc	tcatttttta	accaataggc	cgaaatcggc	aaaatccctt	ataaatcaaa	5520
agaatagccc	gagatagggt	tgagtgttgt	tccagtttgg	aacaagagtc	cactattaaa	5580
gaacgtggac	tccaacgtca	aagggcgaaa	aaccgtctat	cagggcgatg	gcggatcagc	5640
ttatgcggtg	tgaaataccg	cacagatgcg	taaggagaaa	ataccgcata	aggcgctctt	5700
ccgcttcctc	gctcactgac	tcgctgcgct	cggctcgctc	gctgcggcga	gcggtatcag	5760
ctcactcaaa	ggcggtaata	cggttatcca	cagaatcagg	ggataacgca	ggaaagaaca	5820
tgtgagcaaa	caggccagcaa	aaggccagga	accgtaaaaa	ggccgcgttg	ctggcgcttt	5880
tccataggct	ccgccccctc	gacgagcatc	acaaaaatcg	acgctcaagt	cagaggtggc	5940
gaaacccgac	aggactataa	agataaccagg	cgtttcccc	tggaagctcc	ctcgtgcgct	6000
ctcctgttcc	gaccctgccg	cttaccggat	acctgtccgc	ctttctccct	tcgggaagcg	6060
tggcgctttc	tcatagctca	cgctgtaggt	atctcagttc	ggtgtaggtc	gttcgctcca	6120
agctgggctg	tgtgcacgaa	cccccgcttc	agcccgaccg	ctgcgcctta	tccggtaact	6180
atcgtcttga	gtccaacccg	gtaagacacg	acttatcgcc	actggcagca	gccactggta	6240
acaggattag	cagagcgagg	tatgtaggcg	gtgctacaga	gttcttgaag	tgggtggccta	6300
actacggcta	cactagaagg	acagtatttg	gtatctgcgc	tctgctgaag	ccagttacct	6360
tcggaaaaag	agttggtagc	tcttgatccg	gcaaacaac	caccgctggg	agcggcgggt	6420
ttttgtttgc	aagcagcaga	ttacgcgcag	aaaaaaagga	tctcaagaag	atcctttgat	6480
cttttcttac	tgaacgggtga	tccccaccgg	aatt			6514

<210> 2

<211> 5610

<212> DNA

<213> Homo sapien

<400> 2

aaaacttgcg	gccgcggaat	ttcgactcta	ggccattgca	tacgttgat	ctatatcata	60
atatgtacat	ttatattggc	tcattgtccaa	tatgaccgcc	atgttgacat	tgattattga	120
ctagttatta	atagtaatca	attacggggg	cattagttca	tagcccata	atggagtctc	180
gcgttacata	acttacggta	aatggcccgc	ctggctgacc	gcccacgac	ccccgcccat	240
tgacgtcaat	aatgacgtat	gttcccatag	taacgccaat	agggactttc	cattgacgtc	300
aatgggtgga	gtattttacg	taaactgccc	acttggcagt	acatcaagt	tatcatatgc	360
caagtccgcc	ccctattgac	gtcaatgacg	gtaaatggcc	cgcctggcat	tatgccaggt	420
acatgacctt	acgggacttt	cctacttggc	agtacatcta	cgtattagtc	atcgctatta	480
ccatgggtgat	gcgggttttg	cagtacacca	atgggcgtgg	atagcgtttt	gactcacggg	540
gattttccaag	tctccacccc	attgacgtca	atgggagttt	gttttggcac	caaaatcaac	600
gggactttcc	aaaatgtcgt	aataaccccc	ccccgttgac	gcaaatgggc	ggtaggcgtg	660

tacggtggga	ggtctatata	agcagagctc	gttttagtgaa	cogtcagatc	gcctggagac	720
gccatccacg	ctgtttttgac	ctccatagaa	gacaccggga	ccgatccagc	ctccgcggcc	780
gggaacggtg	cattggaacg	cggattcccc	gtgccaaagag	tgacgtaagt	accgcctata	840
gactctatag	gcacaccctt	ttggctctta	tgcatgctat	actgtttttg	gcttggggcc	900
tataaccccc	cgctccttat	gctataggtg	atggtatagc	ttagcctata	ggtgtgggtt	960
attgaccatt	attgaccact	ccoctattgg	tgacgatact	ttccattact	aatccataac	1020
atggctcttt	gccacaacta	tctctattgg	ctatatgcc	atactctgtc	cttcagagac	1080
tgacacggac	tctgtatttt	tacaggatgg	ggtccattta	ttattttacaa	attcacatat	1140
acaacaacgc	cgtcccccg	gcccgcagtt	ttattaaac	atagcgtggg	atctccgaca	1200
tctcgggtac	gtgttcggga	catgggctct	tctccggtag	cggcggagct	tccacatccg	1260
agccctggtc	ccatccgtcc	agcggctcat	ggtcgtcgg	cagctccttg	ctcctaacag	1320
tggaggccag	acttaggcac	agcacaatgc	ccaccaccac	cagtgtgccg	cacaaggccg	1380
tggcggtagg	gtatgtgtct	gaaaatgagc	tcggagattg	ggctcgcacc	tggacgcaga	1440
tggaagactt	aaggcagcgg	cagaagaaga	tcaggcgagc	tgagttgttg	tattctgata	1500
agagtacagag	gtaactcccc	ttgcgggtgct	gtaacgggtg	gagggcagtg	tagtctgagc	1560
agtactcgtt	cgtgcgcgcg	gcgccaccag	acataatagc	tgacagacta	acagactgtt	1620
ctcttccatg	ggtcttttct	gcagtcaccg	tcgtcgacct	aagaattcgc	ccttcgaaac	1680
catgaacttt	ctgctgtctt	gggtgcattg	gagccttgcc	ttgctgtctt	acctccacca	1740
tgccaagtgg	tcccaggctg	cacccatggc	agaaggagga	gggcagaatc	atcacgaagt	1800
ggtgaagtcc	atggatgtct	atcagcgcag	ctactgccat	ccaatcgaga	ccctgggtgga	1860
catcttccag	gagtaccctg	atgagatcga	gtacatcttc	aagccatcct	gtgtgcccct	1920
gatgcgatgc	gggggctgct	gcaatgacga	gggcctggag	tgtgtgcca	ctgaggagtc	1980
caacatcacc	atgcagatta	tgcgatcaa	acctcaccaa	ggccagcaca	taggagagat	2040
gagcttcccta	cagcacaca	aatgtgaatg	cagaccaaag	aaagatagag	caagacaaga	2100
aaatccctgt	gggccttgct	cagagcggag	aaagcatttg	tttgtacaag	atccgcagac	2160
gtgtaaagt	tcttgcaaaa	acacagactc	gcgttgcaag	gcgaggcagc	ttgagttaaa	2220
cgaacgtact	tgcatatgtg	acaagccgag	gcggtgagcc	gggcaggagg	aaggagcctc	2280
cctcaggggt	tcgggaacca	gatctctcac	caggaaagac	tgatacagaa	agggcggaatt	2340
caggcctaag	cttcctaggt	atcgatctcg	agcaagtcta	gaaagccatg	gatatacgat	2400
ccactacgcg	ttagagctcg	tcgatcagcc	tcgactgtgc	cttctagtgt	ccagccatct	2460
gttgtttgcc	cctcccccg	gccttccttg	accctggaag	gtgccactcc	cactgtcctt	2520
tcctaataaa	atgaggaaat	tgcatcgcac	tgtctgagta	ggtgtcattc	tattctgggg	2580
ggtgggggtg	ggcaggacag	caagggggag	gattgggaag	acaatagcag	gggggtgggc	2640
gaagaactcc	agcatgagat	ccccgcgctg	gaggatcatc	cagctagcaa	gtcccatcag	2700
tgatggagtt	ggccactccc	tctctgcgcg	ctcgtctcgt	cactgaggcc	gggcgaccac	2760
aggtgcggcg	acgcccgggc	tttgcccggg	cggcctcagt	gagcagcgca	gcgcgccagc	2820
gattctcttg	tttgctccag	actctcaggc	aatgacctga	tagcctttgt	agagacctct	2880
caaaaatagc	taccctctcc	ggcatgaatt	tatcagctag	aacggttgaa	tatcatattg	2940
atggtgattt	gactgtctcc	ggcctttctc	accggtttga	atctttacct	acacattact	3000
caggcattgc	atttaaaata	tatgaggggt	ctaaaaattt	ttatccttgc	gttgaaataa	3060
aggcttctcc	cgaaaagta	ttacaggggt	ataatgtttt	tgggtacaacc	gatttagctt	3120
tatgctctga	ggctttattg	cttaattttg	ctaattcttt	gccttgccctg	tatgatttat	3180
tggatgttgg	aattcctgat	gcggatattt	ctccttacgc	atctgtgcgg	tatttcacac	3240
cgcatatggt	gcaactctag	tacaatctgc	tctgatgccg	catagttaag	ccagccccga	3300
caccgcgcaa	caccgcgtga	cgcgcctga	cgggcttgct	tgctcccggc	atccgcttac	3360
agacaagctg	tgaccgtctc	cgggagctgc	atgtgtcaga	ggttttcacc	gtcatcaccg	3420
aaacgcgcga	gacgaaaggg	cctcgtgata	cgcctatttt	tataggttaa	tgtcatgata	3480
ataatggttt	cttagacgtc	agggtggcact	tttcggggaa	atgtgcgcgg	aacctctatt	3540
tgttttattt	tctaaataca	ttcaaatatg	tatccgctca	tgagacaata	acctgataa	3600
atgctttaat	aatattgaaa	aaggaagagt	atgagtattc	aacattttccg	tgtcgccctt	3660
attccctttt	ttgcggcatt	ttgccttctc	gtttttgctc	accagaaaac	gctggtgaaa	3720
gtaaaagatg	ctgaagatca	gttgggtgca	cgagtgggtt	acatcgaaact	ggatctcaac	3780
agcggtaaga	tccttgagag	ttttcgcccc	gaagaacggt	ttccaatgat	gagcactttt	3840
aaagtctctg	tatgtggcgc	ggtattatcc	cgtattgacg	ccgggcaaga	gcaactcggc	3900

cgccgcatac	actattctca	gaatgacttg	gttgagtact	caccagtcac	agaaaagcat	3960
cttacggatg	gcatgacagt	aagagaatta	tgcagtgtcg	ccataaccat	gagtataaac	4020
actgcgccca	acttacttct	gacaacgata	ggaggaccga	aggagctaac	cgcttttttg	4080
cacaacatgg	gggatcatgt	aactcgctt	gatacggtggg	aaccggagct	gaatgaagcc	4140
ataccaaacg	acgagcgtga	caccacgatg	cctgtagcaa	tggcaacaac	gttgcgcaaa	4200
ctattaactg	gcgaactact	tactctagct	tcccggcaac	aattaataga	ctggatggag	4260
gcggataaag	ttgcaggacc	acttctgcgc	tcggcccttc	cggtctggctg	gtttattgct	4320
gataaatctg	gagccgggtga	gcgtgggtct	cgcggtatca	ttgcagcact	ggggccagat	4380
ggtaagccct	cccgatctgt	agttatctac	acgacgggga	gtcaggcaac	tatggatgaa	4440
cgaatagac	agatcgctga	gatagggtgcc	tacttgatta	agcattggta	actgtcagac	4500
caagtttact	catatatact	ttagattgat	ttaaaaacttc	atttttaatt	taaaaggatc	4560
taggtgaaga	tcctttttga	taatctcatg	acaaaaatcc	cttaacgtga	gttttcgttc	4620
cactgagcgt	cagaccccg	agaaaagatc	aaaggatctt	cttgagatcc	ttttttctg	4680
cgcgtaatct	gctgcttgca	aacaaaaaaa	ccaccgctac	cagcggtgg	ttgtttgccc	4740
gatcaagagc	taccaactct	ttttccgaag	gtaactggct	tcagcagagc	gcagatacca	4800
aatactgtcc	ttctagtgtg	gccgtagtta	ggccaccact	tcaagaactc	tgtagcaccg	4860
cctacataacc	tcgctctgct	aatcctgtta	ccagtggctg	ctgccagtgg	cgataagtcg	4920
tgtcttaccg	ggttggactc	aagacgatag	ttaccggata	aggcgagcgc	gtcgggtgta	4980
acgggggggt	cgtgcacaca	gccagcttg	gagcgaacga	cctacaccga	actgagatac	5040
ctacagcgtg	agctatgaga	aagcgccacg	cttcccgaag	ggagaaaggc	ggacaggtat	5100
ccggtgaagc	gcaggggtcg	aacaggagag	cgacagagg	agcttccagg	gggaaacgcc	5160
tggatatctt	atagtcctgt	cgggtttcgc	cacctctgac	ttgagcgtcg	atttttgtga	5220
tgctcgtcag	gggggcggag	cctatggaaa	aacgccagca	acgggccttc	tttacggttc	5280
ctggcctttt	gctggccttt	tgtcacatg	ttctttcctg	cgttatcccc	tgattctgtg	5340
gataaccgta	ttaccgcctt	tgagtgaact	gataccgctc	gccgcagccg	aacgaccgag	5400
cgcagcaggt	cagtgaagca	ggaagcggaa	gagcgcccaa	tacgcaaac	gcctctcccc	5460
gcgcgttggc	cgattcatta	atgcagctgg	cgcgctcgct	cgctcactga	ggcgcccg	5520
gcaaagcccg	ggcgtcgggc	gacctttggt	cgcccgccct	cagtgaagca	gcgagcgcgc	5580
agagagggag	tggccaactc	catcactgat				5610

<210> 3

<211> 7096

<212> DNA

<213> Homo sapien

<400> 3

aaaacttgcg	gccgcggaat	ttcgactcta	ggccattgca	tacgtttgtat	ctatatcata	60
atatgtacat	ttatatggc	tcattgtcaa	tatgaccgcc	atggtgacat	tgattattga	120
ctagttatta	atagtaatca	attacgggg	cattagttca	tagcccatat	atggagtcc	180
gcgttacata	acttacggta	aatggccgc	ctggctgacc	gccaacgac	ccccgccat	240
tgacgtcaat	aatgacgtat	gttcccatag	taacgccaat	agggactttc	cattgacgtc	300
aatgggtgga	gtattttacg	taaaactgcc	acttggcagt	acatcaagt	tatcatatgc	360
caagtccgcc	ccctattgac	gtcaatgacg	gtaaatggcc	cgcctggcat	tatgccaggt	420
acatgacctt	acgggacttt	cctacttggc	agtacatcta	cgtattagtc	atcgctatta	480
ccatgggtgat	gcgttttttg	cagtacacca	atgggcgtgg	atagcggttt	gactcacggg	540
gattttccaag	tctccacccc	attgacgtca	atgggagttt	gtttttggc	caaaatcaac	600
gggactttcc	aaaatgtcgt	aataacccc	ccccgttgac	gcaaattggc	ggtaggcgtg	660
tacgggtggga	ggtctatata	agcagagctc	gtttagtga	ccgtcagatc	gcctggagac	720
gccatccacg	ctgtttttgac	ctccatagaa	gacaccggga	ccgatccagc	ctccgcggcc	780
gggaacgggt	cattggaacg	cggattcccc	gtgccaagag	tgacgtaagt	acgcctata	840
gactctatat	gcacacccct	ttggctctta	tgcattgctat	actgtttttg	gcttggggcc	900
tatacacccc	cgcctccttat	gtatatagg	atggtatagc	ttagcctata	ggtgtgggtt	960
attgaccatt	attgaccact	cccctattgg	tgacgatact	ttccattact	aatccataac	1020
atggctcttt	gccacaacta	tctctattgg	ctatatgcca	atactctgtc	cttcagagac	1080

tgacacggac	tctgtatfff	tacaggatgg	gggtccattta	ttattttacaa	attcacatat	1140
acaacaacgc	cgcccccggt	gccccgagtt	tttattaaac	atagcgtggg	atctcgcaga	1200
tctcgggtac	gtgttccgga	catgggctct	tctcggtag	cggcggagct	tccacatccg	1260
agccctggtc	ccatccgtcc	agcggctcat	ggtcgctcgg	cagctccttg	ctcctaacag	1320
tggaggccag	acttaggcac	agcaaatgc	ccaccaccac	cagtgtgccg	cacaaggccg	1380
tggcggtagg	gtatgtgtct	gaaaatgagc	tccgagattg	ggctcgcacc	tggacgcaga	1440
tggaagactt	aaggcagcgg	cagaagaaga	tgcaggcagc	tgagttgttg	tattctgata	1500
agagtcagag	gtaactcccc	ttgcggtgct	gttaacgggtg	gagggcagtg	tagtctgagc	1560
agtactcggt	gctgccgcgc	gcgccaccag	acataatagc	tgacagacta	acagactggt	1620
cctttccatg	ggtcttttct	gcagtcaccg	tgcgcagcct	aagaattcgc	cctttcacca	1680
tggcagcta	ctgggacacc	gggttcctgc	tgtcgcgct	gctcagctgt	ctgcttctca	1740
caggatctag	ttcaggttca	aaattaaaag	atcctgaact	gagtttaaaa	ggcaccacgc	1800
acatcatgca	agcaggccag	acactgcac	tccaatgcag	gggggaagca	gcccataaat	1860
ggctctttgcc	tgaatgggtg	agtaaggaaa	gcgaaaggct	gagcataact	aaatctgcct	1920
gtggaagaaa	tggcaaacaa	ttctgcagta	ctttaacctt	gaacacagct	caagcaaac	1980
acactggctt	ctacagctgc	aaatatctag	ctgtacctac	ttcaagaag	aaggaaacag	2040
aatctgcaat	ctatatattt	attagtata	caggtagacc	tttcgtagag	atgtacagtg	2100
aaatcccgga	aattatcac	atgactgaag	gaaggagct	cgtcattccc	tgccgggtta	2160
cgtcacctaa	catcactggt	actttaaaa	agtttccact	tgacactttg	atccttgatg	2220
gaaaacgcat	aatctgggac	agtagaaagg	gcttcatcat	atcaaatgca	acgtacaaag	2280
aaatagggct	tctgacctgt	gaagcaacag	tcaatgggca	tttgtataag	acaaactatc	2340
tcacacatcg	acaaaccaat	acaatcatag	atgtccaaat	aagcacacca	cgccagtgca	2400
aattacttag	aggccatact	cttgtcctca	attgtactgc	taccactccc	ttgaacacga	2460
gagttcaaat	gacctggagt	taccctgatg	aaaaaaataa	gagagcttcc	gtaaggcgac	2520
gaattgacca	aagcaattcc	catgccaa	tattctacag	tgttcttact	attgacaaaa	2580
tgagaacaa	agacaaagga	ctttatactt	gtcgtgtaag	gagtggaacca	tcattcaaat	2640
ctgttaacac	ctcagtgcac	atatatgata	aagcattcat	cactgtgaaa	catcgaaaac	2700
agcaggtgct	tgaaccgta	gctggcaagc	ggctctaccg	gctctctatg	aaagtgaagg	2760
catttccctc	gccggaagtt	gtatggttaa	aagatggggt	acctgcgact	gagaaatctg	2820
ctcgctatft	gactcgtggc	tactcgttaa	ttatcaagga	cgtaactgaa	gaggatgcag	2880
ggaattatac	aatcttgctg	agcataaaac	agtaaaatgt	gtttaaaaac	ctcactgcca	2940
ctctaattgt	caatgtgaaa	ccccagattt	acgaaaaggc	cgtgtcatcg	ttccagacc	3000
cggctctcta	ccactgggc	agcagacaaa	tcttgacttg	taccgcatat	ggatccctc	3060
aacctacaat	caagtgggtc	tggcaccctt	gtaaccataa	tcattccgaa	gcaagggtgtg	3120
acttttggtc	caataatgaa	gagtccttta	tccctggatgc	tgacagcaac	atgggaaaca	3180
gaattgagag	catcactcag	cgcatggcaa	taatagaagg	aaagaataag	atggctagca	3240
ccttggttgt	ggctgactct	agaatttctg	gaatctacat	ttgcatagct	tccaataaag	3300
ttgggactgt	gggaagaaac	ataagctttt	atatcacaga	tgtgccaaat	gggtttcatg	3360
ttactttgga	aaaaatgccg	acggaaggag	aggacctgaa	actgtcttgc	acagttaaca	3420
agttcttata	cagagacggt	acttggattt	tactgcggac	agttaataac	agaacaatgc	3480
actacagtat	tagcaagcaa	aaaatggcca	tcactaagga	gcactccatc	actcttaatc	3540
ttaccatcat	gaatgtttcc	ctgcaagatt	caggcaccta	tgcttcgaga	gccaggaatg	3600
tatacacagg	ggaagaaatc	ctccagaaga	aagaaattac	aatcagaggt	gagcactgca	3660
acaaaaaggc	tgttttctct	cggatctcca	aatttaaaag	cacaagggaat	gattgtacca	3720
cacaaagtaa	tgtaaaacat	taaaggactc	attaaaaagt	aacagttgtc	tcataatcatc	3780
ttgattttatt	gtcactgttg	ctaactttca	ggctcaaggg	cgaattcagg	cctaagcttc	3840
ctaggtatcg	atctcgagca	agtctagaaa	gccatggata	tcggatccac	tacgcgttag	3900
agctcgctga	tcagcctcga	ctgtgccttc	tagttgccag	ccatctgttg	tttgccctc	3960
ccccgtgcct	tccttgaccc	tggaaagggtg	cactccact	gtcctttcct	aataaaaatga	4020
ggaaattgca	tcgcattgtc	tgagtaggtg	tcattctatt	ctgggggggtg	gggtggggca	4080
ggacagcaag	ggggaggatt	gggaagacaa	tagcaggggg	gtgggcgaag	aactccagca	4140
tgagatcccc	cgcgtggagg	atcatccagc	tagcaagtcc	catcagtgat	ggagttggcc	4200
actccctctc	tgcgcgctcg	ctcgtcact	gaggccgggc	gaccaaagg	cggccgacgc	4260
ccgggctttg	ccggggcggc	ctcagtgcagc	gagcgcggc	gccagcgatt	ctcttgtttg	4320

ctccagactc	tcaggcaatg	acctgatagc	ctttgtagag	acctctcaaa	aatagctacc	4380
ctctccggca	tgaatttatc	agctagaacg	gttgaatatc	atattgatgg	tgatttgact	4440
gtctccggcc	tttctcacc	gtttgaatct	ttacctacac	attactcagg	cattgcattt	4500
aaaatatatg	agggttctaa	aaatttttat	ccttgcggtg	aaataaaggc	ttctcccga	4560
aaagtattac	agggtcataa	tgtttttggg	acaaccgatt	tagctttatg	ctctgaggct	4620
ttattgctta	attttgctaa	ttctttgcct	tgctgtatg	atatttgga	tggtggaatt	4680
cctgatgcgg	tattttctcc	ttacgcatct	gtgcgggtatt	tcacaccgca	tatggtgcac	4740
tctcagtaca	atctgctctg	atgccgcata	gttaagccag	ccccgacacc	cgccaacacc	4800
cgctgacgcg	ccctgacggg	cttgtctgct	cccgccatcc	gcttacagac	aagctgtgac	4860
cgtctccggg	agctgcatgt	gtcagagggt	ttcaccgtca	tcaccgaaac	gcgcgagacg	4920
aaagggcctc	ttatccgcga	tatttttata	ggttaatgtc	atgataataa	tggtttctta	4980
gacgtcaggt	ggcacttttc	ggggaaatgt	gcgcggaaac	cctatttggt	tatttttcta	5040
aatacattca	aatatgtatc	cgctcatgag	acaataaccc	tgataaatgc	ttcaataata	5100
ttgaaaaagg	aagagtatga	gtattcaaca	tttccgtgtc	gcccttatcc	ccttttttgc	5160
ggcatttttg	cttcctgttt	ttgctcacc	agaaacgctg	gtgaaagtaa	aagatgctga	5220
agatcagttg	gggtgcacgag	tggtttacat	cgaactggat	ctcaacagcg	gtaagatcct	5280
tgagagtttt	cgccccgaag	aacgttttcc	aatgatgagc	acttttaaag	ttctgtctatg	5340
tggcgcggta	ttatccgcta	ttgacgcgg	gcaagagcaa	ctcggtcgcc	gcatacctta	5400
ttctcagaat	gacttggttg	agtactcacc	agtcacagaa	aagcatctta	cggatggcat	5460
gacagtaaga	gaattatgca	gtgctgccat	aaccatgagt	gataaactg	cggccaactt	5520
acttctgaca	acgatcggag	gaccgaagga	gctaaccgct	tttttgcaca	acatggggga	5580
tcatgtaact	cgccttgatc	gttgggaacc	ggagctgaat	gaagccatac	caaacgacga	5640
gcgtgacacc	acgatgcctg	tagcaatggc	aacaacgttg	cgcaaaactat	taactggcga	5700
actacttact	ctagcttccc	ggcaacaatt	aatagactgg	atggaggcgg	ataaagtgtc	5760
aggaccactt	ctgcgctcgg	cccttcgggc	tggctggttt	attgctgata	aatctggagc	5820
cgggtgagcgt	gggtctcgg	gtatcattgc	agcactgggg	ccagatggta	agccctcccg	5880
tatcgtagtt	atctacacga	cggggagtca	ggcaactatg	gatgaacgaa	atagacagat	5940
cgctgagata	ggtgcctcac	tgattaaagca	ttggtaactg	tcagaccaag	tttactcata	6000
tatacttttag	attgatttaa	aacttcattt	ttaatttaaa	aggatctagg	tgaagatcct	6060
ttttgataat	ctcatgacca	aaatccctta	acgtgagttt	tcgttccact	gagcgtcaga	6120
cccgtagaa	aagatcaaag	gatcttcttg	agatcctttt	tttctgcgcg	taatctgctg	6180
cttgcaaaaca	aaaaaaaccac	cgctaccagc	ggtggtttgt	ttgccggatc	aagagctacc	6240
aactcttttt	ccgaaggtaa	ctggcttcag	cagagcgcag	ataccaaata	ctgtccttct	6300
agtgtagccg	tagttaggcc	accacttcaa	gaactctgta	gcaccgccta	catacctcgc	6360
tctgtctaac	ctgttaccag	tggctgctgc	cagtggcgat	aagtctgtgc	ttaccgggtt	6420
ggactcaaga	cgatagttac	cggataaggc	gcagcgggtc	ggctgaacgg	ggggttcgtg	6480
cacacagccc	agcttggagc	gaacgacctt	caaccgaactg	agataacctac	agcgtgagct	6540
atgagaaaag	gccacgcttc	ccgaaggag	aaaggcggag	aggtatccgg	taagcggcag	6600
ggtcggaaca	ggagagcgca	cgagggagct	tccaggggga	aacgcctggt	atctttatag	6660
tcctgtcggg	tttcgccacc	tctgacttga	gcgtcgattt	ttgtgatgct	cgtcaggggg	6720
gcggagccta	tggaaaaacg	ccagcaacgc	ggccttttta	cggttcctgg	ccttttctg	6780
gccttttgct	cacatgttct	ttcctgcggt	atccctgat	tctgtggata	accgtattac	6840
cgcctttgag	tgagctgata	ccgctcgccg	cagccgaacg	accgagcgca	gcgagtcagt	6900
gagcgaggaa	gcggaagagc	gccaataacg	caaaccgcct	ctccccgcgc	gttgccgat	6960
tcattaatgc	agctggcgcg	ctcgctcgct	cactgaggcc	gcccgggcaa	agcccgggcg	7020
tcgggcgacc	tttggtcgcc	cggcctcagt	gagcgagcga	gcgcgcagag	agggagtggc	7080
caactccatc	actgat					7096

<210> 4

<211> 636

<212> DNA

<213> Homo sapien

<400> 4

```

atggctccct tagccgaagt cgggggcttt ctggggcgcc tggagggctt gggccagcag      60
gtgggttcgc atttcctgtt gcctcctgcc ggggagcggc cgccgctgct gggcgagcgc      120
aggagcgcgg cggagcggag cgcgcgcggc gggccggggg ctgcgcagct ggcgcacctg      180
cacggcatcc tgcgcgcccg gcagctctat tgccgcaccg gcttcacact gcagatcctg      240
ccgcacggca gcgtgcaggg cacccggcag gaccacagcc tcttcggtat cttggaattc      300
atcagtgtgg cagtgggact ggtcagtatt agaggtgtgg acagtggctc ctatcttggg      360
atgaatgaca aaggagaact ctatggatca gagaaactta cttccgaatg catctttagg      420
gagcagtttg aagagaactg gtataacacc tattcatcta acatatataa acatggagac      480
actggccgca ggtattttgt ggcacttaac aaagacggaa ctccaagaga tggcgccagg      540
tccaagaggc atcagaaatt tacacatttc ttacctagac cagtggatcc agaaagagtt      600
ccagaattgt acaaggacct actgatgtac acttga                                636

```

<210> 5
 <211> 211
 <212> PRT
 <213> Homo sapien

```

<400> 5
Met Ala Pro Leu Ala Glu Val Gly Gly Phe Leu Gly Gly Leu Glu Gly
  1          5          10          15
Leu Gly Gln Gln Val Gly Ser His Phe Leu Leu Pro Pro Ala Gly Glu
          20          25          30
Arg Pro Pro Leu Leu Gly Glu Arg Arg Ser Ala Ala Glu Arg Ser Ala
          35          40          45
Arg Gly Gly Pro Gly Ala Ala Gln Leu Ala His Leu His Gly Ile Leu
          50          55          60
Arg Arg Arg Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Gln Ile Leu
          65          70          75          80
Pro Asp Gly Ser Val Gln Gly Thr Arg Gln Asp His Ser Leu Phe Gly
          85          90          95
Ile Leu Glu Phe Ile Ser Val Ala Val Gly Leu Val Ser Ile Arg Gly
          100         105         110
Val Asp Ser Gly Leu Tyr Leu Gly Met Asn Asp Lys Gly Glu Leu Tyr
          115         120         125
Gly Ser Glu Lys Leu Thr Ser Glu Cys Ile Phe Arg Glu Gln Phe Glu
          130         135         140
Glu Asn Trp Tyr Asn Thr Tyr Ser Ser Asn Ile Tyr Lys His Gly Asp
          145         150         155         160
Thr Gly Arg Arg Tyr Phe Val Ala Leu Asn Lys Asp Gly Thr Pro Arg
          165         170         175
Asp Gly Ala Arg Ser Lys Arg His Gln Lys Phe Thr His Phe Leu Pro
          180         185         190
Arg Pro Val Asp Pro Glu Arg Val Pro Glu Leu Tyr Lys Asp Leu Leu
          195         200         205
Met Tyr Thr
          210

```

<210> 6
 <211> 659
 <212> DNA
 <213> Homo sapien

```

<400> 6
gagcgcagcc ctgatggaat ggatgagatc tagagtggg accctgggac tgtgggtccg      60

```

```

actgctgctg gctgtcttcc tgetgggggt ctaccaagca taccocatcc ctgactccag 120
ccccctctc cagtttgggg gtcaagtccg gcagaggtac ctctacacag atgacgacca 180
agacactgaa gccacctgg agatcagga ggatggaaca gtggtaggcg cagcacaccg 240
cagtcagaa agtctcctgg agtcaaagc cttgaagcca ggggtcattc aaatcctggg 300
tgtcaaagcc tctaggttcc tttgccaaca gccagatgga gctctctatg gatcgctca 360
ctttgatcct gaggcctgca gcttcagaga actgctgctg gaggacggtt acaatgtgta 420
ccagtctgaa gcccatggcc tgcccctgcg tctgcctcag aaggactccc caaaccagga 480
tgcaacatcc tggggacctg tgcgcttcct gcccatgcca ggcctgctcc acgagcccca 540
agaccaagca ggattcctgc cccagagcc cccagatgtg ggctcctctg acccctgag 600
catggtagag cctttacagg gccgaagccc cagctatgcg tcctgactct tcctgaatc 659

```

<210> 7

<211> 210

<212> PRT

<213> Homo sapien

<400> 7

```

Met Glu Trp Met Arg Ser Arg Val Gly Thr Leu Gly Leu Trp Val Arg
 1          5          10          15
Leu Leu Leu Ala Val Phe Leu Leu Gly Val Tyr Gln Ala Tyr Pro Ile
 20          25          30
Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg
 35          40          45
Tyr Leu Tyr Thr Asp Asp Asp Gln Asp Thr Glu Ala His Leu Glu Ile
 50          55          60
Arg Glu Asp Gly Thr Val Val Gly Ala Ala His Arg Ser Pro Glu Ser
 65          70          75          80
Leu Leu Glu Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly
 85          90          95
Val Lys Ala Ser Arg Phe Leu Cys Gln Gln Pro Asp Gly Ala Leu Tyr
100          105          110
Gly Ser Pro His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu
115          120          125
Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro
130          135          140
Leu Arg Leu Pro Gln Lys Asp Ser Pro Asn Gln Asp Ala Thr Ser Trp
145          150          155          160
Gly Pro Val Arg Phe Leu Pro Met Pro Gly Leu Leu His Glu Pro Gln
165          170          175
Asp Gln Ala Gly Phe Leu Pro Pro Glu Pro Pro Asp Val Gly Ser Ser
180          185          190
Asp Pro Leu Ser Met Val Glu Pro Leu Gln Gly Arg Ser Pro Ser Tyr
195          200          205
Ala Ser
210

```

<210> 8

<211> 5974

<212> DNA

<213> Homo sapien

<400> 8

```

aaaacttgcg gccgcggaat ttcgactcta ggccattgca tacgttgtat ctatatcata 60
atatgtacat ttatattggc tcatgtccaa tatgaccgcc atgttgacat tgattattga 120

```

ctagttatta	atagtaatca	attacggggt	cattagttca	tagcccatat	atggagttcc	180
gcgttacata	acttacggta	aatggcccg	ctggctgacc	gcccacgac	ccccgcccat	240
tgacgtcaat	aatgacgtat	gttcccatag	taacgccaat	agggactttc	cattgacgtc	300
aatgggtgga	gtatttacgg	taaactgccc	acttggcagt	acatcaagt	tatcatatgc	360
caagtccgcc	ccctattgac	gtcaatgacg	gtaaatggcc	cgctggcat	tatgccagtc	420
acatgacctt	acgggacttt	cctacttggc	agtacatcta	cgtattagtc	atcgctatta	480
ccatgggtgat	gcggttttgg	cagtacacca	atgggcgtgg	atagcggttt	gactcacggg	540
gattttccaag	tctccacccc	attgacgtca	atgggagttt	gttttggcac	caaaatcaac	600
gggacttttc	aaaatgtcgt	aataaccccc	ccccgttgac	gcaaattgggc	ggtaggcgtg	660
tgacgtggga	ggtctatata	agcagagctc	gtttagtga	ccgtcagatc	gcctggagac	720
gccatccacg	ctgttttgac	ctccatagaa	gacaccggga	ccgatccagc	ctccgcggcc	780
gggaacgggtg	cattggaacg	cggaattcccc	gtgccaagag	tgacgtaagt	accgcctata	840
gactctatag	gcacacccct	ttggctctta	tgcatgctat	actgtttttg	gcttggggcc	900
tatacacccc	cgctccttat	gctatagggtg	atgggtatagc	ttagcctata	ggtgtgggtt	960
attgaccatt	attgaccact	cccctattgg	tgacgatact	ttccattact	aatccataac	1020
atggctctct	gccacaacta	tctctattgg	ctatatgcca	atactctgtc	cttcagagac	1080
tgacacggac	tctgtatttt	tacaggatgg	gttccattta	ttatttacia	attccacat	1140
acaacaacgc	cgcccccg	gcccgcagtt	tttattaaac	atagcgtggg	atctccgaca	1200
tctcgggtac	gtgttcggga	catgggctct	tctccggtag	cgccggagct	tccacatccg	1260
agccctggtc	ccatccgtcc	agcggctcat	ggtcgctcgg	cagctccttg	ctcctaacag	1320
tggaggccag	acttaggcac	agcacaatgc	ccaccaccac	cagtgtgccc	cacaaggccg	1380
tggcggtagg	gtatgtgtct	gaaaatgagc	tcggagattg	ggctcgcacc	tggacgcaga	1440
tggaagactg	aaggcagcgg	cagaagaaga	tgaggcagc	tgagttgttg	tattctgata	1500
agagtcagag	gtaactccc	ttgcgggtgct	gttaacgggtg	gagggcagtg	tagtctgagc	1560
agtactcgtt	gctgcgcgc	gcgccaccag	acataatagc	tgacagacta	acagactgtt	1620
cctttccatg	ggtcttttct	gcagtcaccg	tcgtcgacct	aagaattcag	gtatggctgc	1680
tggttctatc	actaccctgc	cagctctgcc	agaagacggg	ggttctgggtg	ccttcccacc	1740
aggctcacttc	aaagacccaa	aacgtctgta	ctgcaaaaaac	ggtggtttct	tcctgcgcac	1800
ccaccccgac	ggccgagtg	acggggctcc	cgagaagagc	gacccacaca	tcaaactaca	1860
acttcaagca	gaagagagag	gggttggtgc	tatcaaagga	gtgtgtgcaa	accgttacct	1920
tgctatgaaa	gaagatggaa	gattactagc	ttctaaatgt	gttacagacg	agtgtttctt	1980
ttttgaacga	ttggagtcta	ataactacia	tacttacccg	tcaaggaaat	acaccagttg	2040
gtatgtggca	ctgaaacgaa	ctgggcagta	taaacttggg	tccaaaacag	gacctgggca	2100
gaaagctata	ctttttcttc	caatgtctgc	taagagctga	tcttaatggc	agcatctgat	2160
ctcattttac	atgaagcttc	ctaggtatcg	atctcgagca	agtctagaaa	gccatggata	2220
tcggatccac	tacgcgttag	agctcgctga	tcagcctcga	ctgtgccttc	tagttgccag	2280
ccatctgttg	tttgcccttc	ccccgtgcct	tccttgaccc	tggaaagggtc	cactcccact	2340
gtcctttcct	aataaaatga	ggaaattgca	tcgcattgtc	tgagtaggtg	tcattctatt	2400
ctgggggggtg	gggtggggca	ggacagcaag	ggggaggatt	gggaagacaa	tagcaggggg	2460
gtgggcgaag	aactccagca	tgagatcccc	gcgctggagg	atcatccagc	tagcaagtcc	2520
catcagtgat	ggagttggcc	actccctctc	tgccgcctcg	ctcgctcact	gaggccgggc	2580
gaccaaagg	cgcccgacgc	ccgggctttg	cccgggcggc	ctcagtgagc	gagcgagcgc	2640
gccagcgatt	ctcttgtttg	ctccagactc	tcaggcaatg	acctgatagc	ctttgttagag	2700
acctctcaaa	aatagctacc	ctctccggca	tgaatttatc	agctagaacg	gttgaatc	2760
atattgatgg	tgatttgact	gtctccggcc	ttctccacc	gtttgaatct	ttacctacac	2820
attactcagg	cattgcattt	aaaatatatg	agggttctaa	aaatttttat	ccttgcgttg	2880
aaataaaggc	ttctcccgca	aaagtattac	agggtcataa	tgtttttggg	acaaccgatt	2940
tagctttatg	ctctgaggct	ttattgctta	attttgcata	ttctttgcct	tgctgtatg	3000
atattattgga	tggttggaatt	cctgatgcgg	tattttctcc	ttacgcactc	gtgcgggtatt	3060
tcacacgcga	tggtgtgcac	tctcagtaca	atctgctctg	atgccgcata	gttaagccag	3120
ccccgacacc	cgccaacacc	cgctgacgcg	ccctgacggg	cttgtctgct	cccgcatcc	3180
gcttacagac	aagctgtgac	cgtctccggg	agctgcatgt	gtcagaggtt	ttcaccgtca	3240
tcaccgaaac	gcgcgagacg	aaagggcctc	gtgatacgcc	tatttttata	ggttaatgtc	3300
atgataataa	tggtttctta	gacgtcaggt	ggcacttttc	ggggaaatgt	gcgcggaacc	3360

cctatttgtt	tatttttcta	aatacattca	aatatgtatc	cgctcatgag	acaataaccc	3420
tgataaatgc	ttcaataatg	taccogtcaa	gaaggcgata	gaaggcgatg	cgctgcgaat	3480
cgggagcggc	gataccgtaa	agcâcagagga	agcggtcagc	ccattcgctt	cagcaatatac	3540
acgggtagcc	aacgctatgt	cctgatagcg	gtccgccaca	cccagccggc	cacagtcgat	3600
gaatccagaa	aagcggccat	tttccaccat	gatattcggc	aagcaggcat	cgccatgggt	3660
cacgacgaga	tctcgcgcgt	cgggcatgcg	cgccttgagc	ctggcgaaca	gttcggctgg	3720
cgcgagcccc	tgatgctctt	cgtccagatc	atcctgatcg	acaagaccgg	cttccatccg	3780
agtacgtgct	cgctcgatgc	gatgtttcgc	ttgggtggcg	aatgggcagg	tagccggatc	3840
aagcgtatgc	agccgcgcga	ttgcatcagc	catgatggat	actttctcgg	caggagcaag	3900
gtgagatgac	aggagatcct	gccccggcac	ttcggccaat	agcagccagt	cccttcccgc	3960
ttcagtgaca	acgtcgagca	cagctgcgca	aggaacgccc	gtcgtggcca	gccacgatag	4020
ccgcgctgcc	tcgtcctgca	gttcattcag	ggcaccggac	aggtcggctc	tgacaaaaag	4080
aaccggggcg	ccctgcgctg	acagccggaa	cacggcggca	tcagagcagc	cgattgtctg	4140
ttgtgcccag	tcatagccga	atagcctctc	cacccaagcg	gccggagaac	ctgcgtgcaa	4200
tccatcttgt	tcaatcatgc	gaaacgatcc	tcatacctgc	tcttgatcag	atcttgatcc	4260
cctgcgcctc	cagatccttg	gcggcaagaa	agccatccag	tttactttgc	agggcttccc	4320
aaccttacca	gagggcgccc	cagctggcaa	ttcgggttcg	cttgctgtcc	ataaaaccgc	4380
ccagtctagc	tatcgccatg	taagcccact	gcaagctacc	tgctttctct	ttgcgcttgc	4440
gttttccctt	gtccagatag	cccagtagct	gacattcctc	cggggtcagc	accgtttctg	4500
cggactggct	ttctacgtgt	tccgcttcct	ttagcagccc	ttgcgccctg	agtgcttgcg	4560
gcagcgtgaa	gctgtcaatt	ccgcgttaaa	tttttgttaa	atcagctcat	tttttaacca	4620
ataggccgaa	atcggcaaaa	tcccttataa	atcaaaagaa	tagcccagaa	taggggttag	4680
tgttggtcca	gtttggaaca	agagtccact	attaaagaac	gtggactcca	acgtcaaagg	4740
gcgaaaaacc	gtctatcagg	gcgatggcgg	atcagcttat	gcgggtgtaa	ataccgcaca	4800
gatgcgtaag	gagaaaaatac	cgcatacagg	gctcttcgcg	ttcctcgtct	actgactcgc	4860
tgcgctcggg	cgttcggctg	cggcgagcgg	tatcagctca	ctcaaaggcg	gtaatacggg	4920
tatccacaga	atcaggggat	aacgcaggaa	agaacatgcg	gcgcgccaca	tgtgagcaaa	4980
aggccagcaa	aaggccagga	accgtaaaaa	ggccgcgttg	ctggcgtttt	tccataggct	5040
ccgccccctc	gacgagcatc	acaaaaatcg	acgctcaagt	cagagggtgg	gaaacccgac	5100
aggactataa	agataccagg	cgtttccccc	tggaagctcc	ctcgtgcgct	ctcctgttcc	5160
gaccctgcgg	cttaccggat	acctgtccgc	ctttctccct	tcgggaagcg	tggcgctttc	5220
tcatagetca	cgctgtaggt	atctcagttc	gggtgtaggtc	gttcgctcca	agctgggctg	5280
tgtgcacgaa	ccccccgctc	agcccagacc	ctgcgcctta	tccggtaact	atcgtcttga	5340
gtccaacccg	gtaagacacg	acttatcgcc	actggcagca	gccactggta	acaggattag	5400
cagagcgagg	tatgtaggcg	gtgctacaga	gttcttgaag	tggtggccta	actacggcta	5460
cactagaagg	acagtatttg	gtatctgcgc	tctgctgaag	ccagttacct	tcggaaaaag	5520
agttggtagc	tcttgatccg	gcaaacaaac	caccgctggg	agcggcggtt	ttttgtttgc	5580
aagcagcaga	ttacgcgcag	aaaaaaâgga	tctcaagaag	atcctttgat	cttttcttac	5640
tgaacggtga	tccccaccgg	aattgcggcc	catgttcttt	cctgcgttat	cccctgattc	5700
tgtggataac	cgtattaccg	cctttgagtg	agctgatacc	gctcgcgcga	gccgaacgac	5760
cgagcgcagc	gagtcagtga	gcgaggaagc	ggaagagcgc	ccaatacgca	aaccgcctct	5820
ccccgcgcgt	tggccgattc	attaatgcag	ctggcgcgct	cgctcgctca	ctgagggcgc	5880
ccgggcaaag	cccgggcgtc	gggcgacctt	tggtcgcccc	gcctcagtga	gcgagcgagc	5940
gcgcagagag	ggagtggcca	actccatcac	tgat			5974

<210> 9

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used for PCR amplification

<400> 9

ggtattttaa acttgcggcc gcggaatttc gactctaggc c 41

<210> 10

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide used for PCR amplification

<400> 10

gctgcccgga acttgctagg tggatgatcc tccagcgagg ggatctcatg 50

<210> 11

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 11

agatataagc ttaccatggg tgaaaagcgt ctgccccca aa 42

<210> 12

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer

<400> 12

cgcgcgctcg agaccatgag gaatattatc caaagcgaaa ct 42

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.